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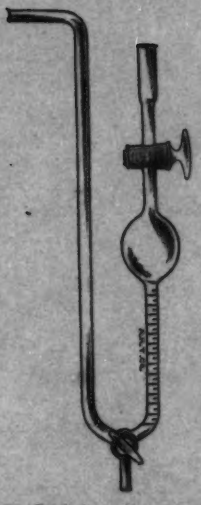

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UNILATERAL DESTRUCTION OF THE SEMICIRCULAR CANALS IN CATS

HOMER G. FISHER AND HENRY R. MULLER
From the Anatomical Laboratory of the Johns Hopkins University

Received for publication June 13, 1916

In a previous paper (3), dealing with the physiological anatomy of the falling reflex of cats, a number of observations upon cats subjected to a unilateral removal of the semicircular canals were reported. These former observations were all made as acute experiments, and the rotatory reaction was tested on recovery from ether. In order to determine the permanence of such a lesion, and to ascertain a possible compensation due to assumption of function by other organs, a series of cats was prepared with similar unilateral destruction of the semicircular canals. These observations were continued in some cases over a period of approximately three months; it is the purpose of this paper to present the records of these animals.

Although many workers in physiology have been concerned with the function of the semicircular canals in lower animals (notably pigeons), similar recorded experiments in the form of unilateral destruction of the labyrinth of mammals have been few. Several observations extending over a considerable period of time have been made upon birds and amphibia chiefly. Pigeons, several months after the destruction of one labyrinth, according to Ewald (2), are distinguished with difficulty from normal pigeons. As they are able to perform perfectly their ordinary functions, special tests must be used in order to bring out the slight permanent changes, such as the position of the head, and the preference for standing on the leg of the non-operated side. In regard to frogs with only a single intact canal-system, the same author mentions the fact that the peculiar rotation of the head remains for a long time, decreasing only in its intensity as time goes on. When, however,

the frog is disturbed, the original abnormal position is assumed again in its former degree. In some as yet unpublished work by Rosencrantz (4), pigeons with the three canals on one side cut, still showed after a period of six and a half months, some slight residuals of the original lesion; there was not an absolutely complete recovery. Wilson and Pike (6) report that in mammals, e.g., dogs and monkeys, upon the removal of the semicircular canals of one side, certain postural phenomena only, such as the torsion of the head to the injured side and a leaning, particularly of the anterior part of the body to this side, remain permanently.

Since it is not practicable to reach the labyrinth by the same method in every species of animal, many different methods for doing so have been devised. For these experiments the procedure of Wilson and Pike for destroying the canals in cats was adopted, with the modification, however, that a median, dorsal, longitudinal skin incision was made, the superior auricular muscle reflected, and the origin of the temporal muscle laid back from the bone, so as better to preserve the function of these muscles. The cats were operated upon under full ether anaesthesia, strict surgical cleanliness was observed, and the wound was repaired by suturing with silk thread. In the spacious cages in which the cats were kept after the operation, ample opportunity for walking, jumping and climbing was provided.

Before detailing the results noted, it will be well to make clear the terms used in describing the turning reactions, i.e., "turn to the right," and "turn to the left." By saying that a cat turns to the right, for instance, is meant, that if the cat were standing upright on its hind feet it would turn on its long axis in the same direction that a man would turn when turning to his right. Similarly with the term, "turn to the left." Or, in other words, when the cat is standing on its four feet, or turns in the air starting from a position with its feet directed upward, a "right turn" would be one in which, looking at the cat from the cephalic end, the turn of the body would take place in a clockwise direction around the long axis of the body; in a "turn to the left" the turn would be in an anti-clockwise direction. When it is said that an animal "falls on its right side," or "towards the right side," the animal in doing so really turns to the left around its long axis.

Directly after the operation, upon complete recovery from the ether, the reactions of the cats with destruction of the semicircular canals on one side corresponded exactly with those noted in the previous paper. There was a horizontal nystagmus of both eyes to the

opposite side, a partial rotation of the head away from the lesion, and an ataxia, especially of the head, variable in degree. The animals reclined always upon the operated side, resisting strongly rotation away from the lesion. Upon being dropped they rotated in the air so as to land upon their feet normally, although requiring a greater distance in which to inaugurate and also to perform the rotatory movement. The turn was always to the right with left-sided lesions, and to the left with right-sided lesions. The majority of animals immediately after recovery from anaesthesia will not rotate in the air while blinded, perhaps because they have not yet learned to trust the impressions received from the intact labyrinth.

The picture presented by one of these cats eighteen to twenty-four hours after the operation was quite constant. The animal showed little desire to walk, but when it did so, it either went in a circle towards the side operated upon; or when it attempted to walk in a straight line, its legs were noticeably abducted, more markedly on the side not operated on than on the affected side. During this progression the whole side of its body was held much lower on the operated side than on the other. This sagging suggested a weakness or loss of tonicity of the muscles on the affected side. Very often, in walking, the animal suddenly fell towards its affected side, i.e., if the right side were the side that had been operated upon, the body would turn to the left around the long axis; and vice versa. It did not, however, tend to continue rolling over and over about its long axis across the floor as Ewald's rabbits did with similar lesions, but it soon got on its feet again and started to walk. However, after taking a few more steps forward it usually fell over on its side again, invariably to the side of the lesion. It was frequently noted that when such a cat, sitting or standing quietly, suddenly shook its head rapidly, the whole body would suddenly fall over towards the affected side. This phenomenon could be brought out also by shaking the head of the animal rapidly from side to side for a few seconds. When the shaking was stopped the animal immediately fell over on its side.

When an animal with a unilateral vestibular lesion was allowed to jump off a chair it would land fairly accurately on its feet, but immediately upon striking the floor the body of the animal would fall over to the side on which the canals had been destroyed. Upon regaining its feet the animal would then start to walk off either to the right or to the left respectively, depending upon whether the right or the left canals had been operatively interfered with.

The attitude of the head of the animal while sitting or standing quietly or while walking was very characteristic and corresponded exactly to the position of the head assumed by frogs as pictured by Ewald, and by mammals in Wilson and Pike's article. The head is rotated around the long axis of the body so that the ear on the affected side is inclined downward, and the nose directed over towards the sound side. This attitude was never changed and was increased to a considerable extent when the cat lapped milk or performed other tasks requiring muscular effort. It was sometimes increased to such an extent in these instances that the side of the head with the lesion was held parallel with the floor.

All the animals still had a horizontal nystagmus to a varying degree after eighteen hours from the time of operation. At times it was rapid, and at times it was slower and coarser. The quick phase of the nystagmus was always towards the non-operated side. Invariably, however, after twenty-four to thirty-six hours, it had completely disappeared.

The responses obtained from a cat with one intact labyrinth to dropping through the air upon a soft bed of straw were very consistent. In every case the animal was first held with its back down and its feet pointing straight upward. If it were an animal, for example, with the right labyrinth removed, rotation was always to the left; the landing on its feet was quite accurate. If this same animal were held so that its feet were in a horizontal plane, with the side of its body and head on which the labyrinth was destroyed (that is, the right in this case) parallel with the floor, it twisted through 270 degrees or more to the left in order to land on its feet. Sometimes it turned too far, going through more than 270 degrees and landing on the side of its body. In every case, however, it made the rotation to the left. Animals with a left-sided lesion reacted in a similar manner, except that they invariably turned to the right. Hence it must be assumed that these experimental cats, on falling, always turned away from the lesion.

When blinded by a hood placed over the head, cats, with one-sided lesions created twenty-four hours or more previously, reacted by turning in the same way as when not blinded. Such a rotation of blinded cats rarely occurs immediately after the operation, but in the short period of twenty-four hours the animal apparently learns to rely upon impulses derived from the one intact labyrinth. With the lesions on the right side in such an animal the turn was always to the left, and vice versa (even if by doing so it was necessary to go through more than 270 degrees in order to secure a perfect landing). But now it was also

seen that such animals very often—much more frequently than the non-blinded cats—went too far in their twisting, striking the straw on their sides. Vision, of course, as recorded in the former paper, aided the animal to determine accurately its position in space. One cat when blinded, was seen to make two complete revolutions (720 degrees) through the air before striking the straw. Such extreme rotations require considerable heights for their accomplishment.

The acquirement of the ability of a cat with but one intact vestibule to rotate in the air, on falling, even when blinded is but one indication of a gradual new adaptation of such an organism to its environment. In this instance the recovery of function on the part of one canal-system took place invariably within twenty-four hours. A more gradual recovery was noted in the muscular reactions of all of these cats with one-sided lesions. As was recorded in the previous note, a cat with a unilateral destruction of the semicircular canals required a fall of a considerable distance before the normal rotation was started, if the test were made as an acute experiment shortly after the animal had recovered from the surgical anaesthesia. In these observations, however, the cats twenty-four hours after the operation were noticed to have acquired the power of inaugurating the rotatory response in much more rapid a manner. Thus a fall of 2 to 3 feet was sufficient to allow them to make the necessary twist through 180 degrees. Further acquirement of this ability was a rather slow process, and it was not until two to three weeks after the operation that these animals showed an almost total recovery of their ability to initiate the typical rotation within a very short distance. None of the animals, however, during the three months' duration of observation made a complete recovery. The best explanation of this slow recovery probably concerns not the acquirement of a more acute function on the part of the receptor system, but rather deals with a gradual, but never wholly complete, resumption of normal motor function. Such an explanation would undoubtedly coincide with Ewald's original dictum concerning the relation of the vestibular apparatus to muscular tonus.

With the return, then, of more or less muscular reaction, the rotation of a blinded cat with a one-sided destruction of the vestibule could be foreseen as an example of the power, and possibly of the need, of such an animal to maintain a continuous revolution about the long axis of the body. As recorded above, such a cat was observed to rotate through 720 degrees in the course of a fall of moderate height. Presumably this animal on a much greater fall would have maintained a contin-

uous rotation about its long axis, differing in this regard from the animal with the more acute lesion in its greater muscular power and coordination. This suggests that cats when deprived of their ocular sensations and with but one intact vestibule possess an inherent tendency towards this rotation about the long axis. But another factor undoubtedly influences such continuous revolution of the animal. This factor probably concerns the character of the physical environment to which the animal is immediately subjected. For if such a blinded animal be placed upon the ground, the inevitable reaction consists not in the rotation but solely in a struggle to remove the blinding hood. Deprived, however, of its firm and customary basis for support, as in the air, the single unbalanced canal-system exerts an overpowering and almost inevitable stimulus for the rotation about the long axis of the body. Such a rotation, however, in an unblinded cat seems limited merely to the necessary turning which will protect the animal from injury and allow it to land squarely on its feet. Thus it may be assumed on this hypothesis that a blinded cat with a unilateral destruction of one set of semicircular canals possesses a tendency towards the rotation about its long axis only when in an unstable medium, such as obtains on falling through the air.

This hypothesis was tested by placing the unilateral cats in another unstable medium—water. Five cats, not blinded, two with right lesions, and three with left lesions, were placed in a tub of water, deep enough for them to swim around in without having their feet touch bottom. The results were very striking in that they all started to swim, but after a few strokes the side of the body with the lesion went down in the water and the other side came up. In doing this their heads became submerged, which deprived them of the use of the eyes, and now they began to revolve continuously around the long axis—to the left in those with right-sided lesions, and to the right in those with left-sided lesions. This rapid, continuous revolving of the body lasted as long as the animals were permitted to remain in the water. Interesting was the fact that two of these animals, 59 and 67 days respectively after the operation, were able to swim and climb out of the tub as long as they kept their heads above water; as soon as the heads became submerged they began revolving about the long axis. In this connection Wilson and Pike report in a protocol, that a dog which had had the right labyrinth destroyed a year previously, when taken out to swim, "sometimes turned over and over in the water before starting, but usually came to the surface when thrown in and started for the bank without rotation or turning in a circle."

In the foregoing paragraphs some mention has been made of the gradual change in reaction of these cats with unilateral destruction of the labyrinth. Thus, the failure of the nystagmus to persist for over thirty hours after the creation of the lesion, was recorded. The tendency to walk about in a circle has been observed to persist only for the first few days. Likewise the gradual return of an almost complete muscular ability was pointed out. Certain reactions, however, do seem to endure unchanged at least throughout the period of three months. These reactions concern the inevitable tendency of the animal to rotate away from the lesion about the long axis when placed in an unstable medium. The other characteristic phenomena of such an animal deprived of one labyrinth are chiefly postural in character, and these, in a way similar to the gradual return of the muscular power, come to approximate the normal, but in no sense must this return be considered as a total resumption of the normal functions. Thus, the sagging of one side of the body on walking is gradually overcome but never completely. The animals seemingly tend to lean towards the operated side. In the same category must be included the abnormal position of the head with the depression of the ear towards the operated side, and the incomplete rotation of the nose. This persists probably to a greater extent, and for a longer period than does the loss of muscular power. In every case a very considerable vestigial remnant of this postural reaction was evident three months after the initial lesion. Interesting in this connection is the fact that on exertion or during any special effort, this abnormal deflection of the head would reappear and persist to a marked degree, but for merely a few moments. It was noted that shortly after the initial operation these animals when shaking their heads fell towards the affected side. Gradually, in the course of several weeks, this spontaneous phenomenon grew less and less frequent and the tendency to fall less and less pronounced. So it would seem that the animals subjected to a unilateral destruction of the semicircular canals tend slowly to recover from the initial effects of such a lesion; the recovery in no sense can be taken to be complete.

SUMMARY

In conclusion it may be stated that cats subjected to a unilateral destruction of the semicircular canals show the following typical responses:

1. During the first twenty-four hours there results a marked horizontal nystagmus to the opposite side; a varying degree of ataxia of the

whole body (especially of the head); and a typical rotation of the head with depression of the ear on the operated side.

2. With the loss of the nystagmus after about thirty hours, and a loss of the tendency to walk in a circle after a few days, there is a very gradual, but incomplete recovery of the postural and muscular reactions of the animal.

3. On being dropped in the air, the tendency of these animals is to rotate away from the lesion, and to land accurately on their feet.

4. Blinded cats with one-sided vestibular lesions show after the first twenty-four hours an inevitable tendency towards rotation away from the lesion about the long axis of their bodies. Such rotations have been observed to continue through 720 degrees. Similarly, unblinded cats when placed in water, and with their heads submerged, continuously rotate around the long axis of their bodies.

5. All the rotations, whether falling to the injured side while walking, or rotating while dropping through the air, or rotating while submerged in water, all take place in the same direction around the long axis of the body—to the right in cats with the lesion on the left side, and to the left in those with right-sided lesions.

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A PHOTOGRAPHIC METHOD FOR MEASURING THE SURFACE AREA OF THE HUMAN BODY

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From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.

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In a study made at the Nutrition Laboratory in 1912 of the metabolism of a man during a 31-day fast, the heat production was calculated in accordance with the usage of physiologists on the basis of per kilogram of body weight and also on the basis of per square meter of body surface (1). As the only method available at that time for computing the body surface was the Meeh formula, i.e., 12.312 $\sqrt[3]{\text{body weight}^2}$, it was used in this instance. In the discussion of the results, however, it was stated that owing to probable defects in the applicability of the Meeh formula the absolute values were doubtless erroneous and that even the differences could not be looked upon as entirely accurate. With the development of the Du Bois linear formula (2) for measuring the body surface and the consequent demonstration of errors in the Meeh formula, an effort was made to obtain more probable values for the body surface of the fasting man for subsequent use in recomputing the heat production per square meter of body surface. Unfortunately the measurements made of the subject during the experimental period i.e., the regular anthropometric measurements of the gymnasium, are not those required by the Du Bois formula. Use was therefore made of a series of anatomical photographs of the subject taken at intervals during the fast and a photographic method was developed for measuring the body surface which was based entirely upon the Du Bois linear formula. We were thus able indirectly to take advantage of the greater accuracy of the Du Bois method. The results of this recomputation of the body surface of the fasting man are published in the accompanying article (3), together with a discussion of the heat production per square meter of body surface as computed upon the new basis.

This photographic method for measuring the body surface of indi-

viduals was the outcome of an attempt previously made to secure the body surface of infants. At that time certain photographic poses were studied with a view to using them as a possible basis of measurement of the body area of adults (4), but with the Du Bois linear formula it was practicable to simplify these poses considerably. In the development of the photographic method photographs were taken of a considerable number of normal individuals of widely varying weight and body configuration whose body area had been measured by means of the Du Bois formula. The areas shown in these photographs were then accurately measured by the planimeter. From the results obtained with the planimeter and the values for the body surface of the individuals as secured by actual measurement and computation with the Du Bois formula, a formula was developed which could be used for calculating the body surface of any individual from photographs taken of certain poses. A detailed description of the development of the method and the values obtained for the individuals photographed are given in this paper.

GENERAL PRINCIPLE OF THE PHOTOGRAPHIC MEASUREMENTS

As a basic measurement a cross section of the nude body through the extended arms and fingers, trunk and legs was considered to be the closest approximation to the body surface. If the body were as flat as a leaf this area, multiplied by 2, would give the total body surface but the impractical nature of even such a cross section as this is instantly seen when it is remembered that while the cross section of the extended hands and fingers is included in the surface area, the cross section of the feet and toes is not included since they are at right angles to the body. This cross section would furthermore assume equality in surface area on the dorsal and ventral sides of the body. With approximately normal men this would not be too gross an assumption but the mammae in women and the pendulous abdomen of the obese would not be included; hence a profile view was taken with the subjects standing at "attention." Various other profile views were taken with one arm extended and with and without one leg forward of the other to include greater lateral area. A more detailed description of the principal poses, with illustrations, will be given later.

It must be noted at the outset that these photographs are made in a wholly empirical way for obviously the human body is not as flat as a leaf and likewise the profile view does not represent the thickness of

the arm but shows only a cross section through the center of the body. Nevertheless, since for comparison the actual body surface could be computed by the Du Bois linear formula from definite body measurements, it seemed feasible to attempt some correlation between the body areas of a number of individuals as measured by the planimeter on photographs showing these poses.

TECHNIQUE

Inasmuch as the outline of the body alone was desired, no attempt was made to differentiate sharply the details of the photographs, particularly as a quick exposure was highly advantageous. The technique for taking the first photographs conformed to the technique employed with the anatomical photographs of the fasting subject. In these a black background was used in the belief that the predominantly light-colored body would give a sharp contrast. Reasonably satisfactory results were obtained in the series of photographs taken in this way although certain dark shadows, particularly on the insides of the legs and portions of the side of the body made it difficult to trace the outline with the planimeter.

The best results were obtained by using as a background a white, semi-transparent screen (a bed sheet) which was placed in front of a window. The camera was located approximately 3 meters from the body and pointed directly toward the window. This procedure gave a sharp black outline against a white background, the resulting print being a fairly good silhouette. But even with this method a few of the pictures showed certain high lights on the shoulders which led us to believe that it would be better to restrict the white field to correspond closely to the form of the body. Theoretically the more nearly the outline on the photograph represents a true reduction of the silhouette the better. To give a suitable scale for measurement, a meter stick was always placed near the body in the same plane as the silhouette and included in the photograph.

Practically all of our pictures were taken with a lens already in the possession of the laboratory, i.e., a Cooke lens, Series V, f/8, $6\frac{1}{2}$ x $8\frac{1}{2}$ inches, with an equivalent focus of 11.2 inches. This was used with a $6\frac{1}{2}$ x $8\frac{1}{2}$ inch plate, the body nearly covering the plate. A series of smaller photographs, taken with a relatively inexpensive Kodak on 4 x 5 inch plates, was also made of one of the subjects. These latter photographs, although requiring more care in planimetering, gave con-

cordant results with the photographs obtained on the large plates. To study the effect of possible distortion, an extraordinarily wide angle lens—a Bausch and Lomb, Zeiss Protar, f/18, Series V, $6\frac{1}{2}$ x $8\frac{1}{2}$ inch, with an equivalent focus of $5\frac{9}{16}$ inches was used with a $6\frac{1}{2}$ x $8\frac{1}{2}$ inch plate. Even when these distorted pictures were planimetered, values were obtained which did not exceed 9 per cent of those found with the Cooke lens, the values ranging from 3 to 9 per cent. Such an extraordinarily wide angle lens would rarely be used, however, as the photographer would instantly see the distortion. Consequently we may say that with practically any lens giving figures not evidently distorted a high degree of accuracy and concordance can be obtained. The prints were made on carbon Velox paper and when dry were temporarily mounted on a drawing board with thumb tacks; the area was then planimetered.

POSES

The photographs were taken of the subject in three principal poses, which are here designated as poses A, B, and C. Later developments showed that of these pose C is the most useful and accordingly, to illustrate the exact method of taking the pictures, a typical photograph of this pose is given of subject 7 (fig. 1). Furthermore, silhouettes of the same subject in all three poses are given (fig. 2), together with silhouettes of poses A and C for a normal woman subject, no. 17, a very fat man, no. 1, and a very small man, no. 14 (fig. 3). Silhouettes are also given of pose C for all of our other subjects (fig. 4). The scale is indicated in each case by a line showing the length of the meter stick photographed at the same time in the same plane as the part of the body focused upon, i.e., in poses B and C exactly opposite the backbone. To destroy the identity of the subjects, the contour of the face has been obliterated in all of the silhouettes.

Pose A. On the supposition that pose A, representing presumably the largest regular cross-section of the body, would be more particularly the basic measurement to which could be added a factor of possibly varying value, special emphasis was laid upon it and a large number of photographs were taken showing the hands and arms in various positions. At first the hands and arms were extended with the open hand at approximately the level of the ear, the arms spread as nearly as possible in the plane of the median line of the body. Subsequently it was found that an easier pose and one that gave exactly the same area when measured by the planimeter was that illustrated, i.e., a dorsal

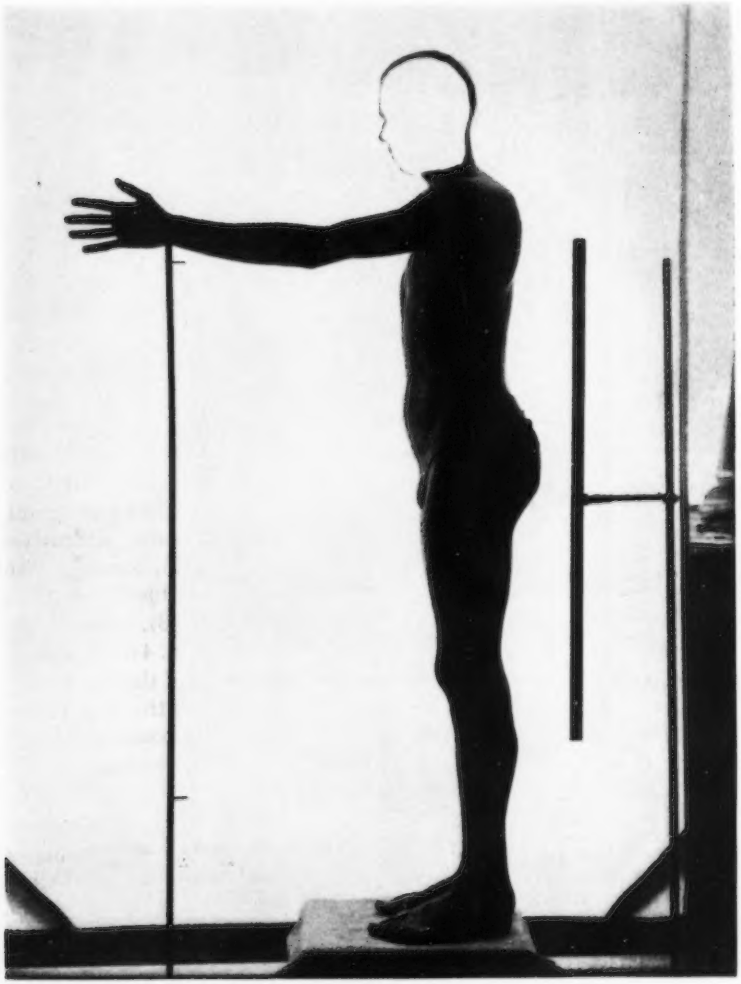
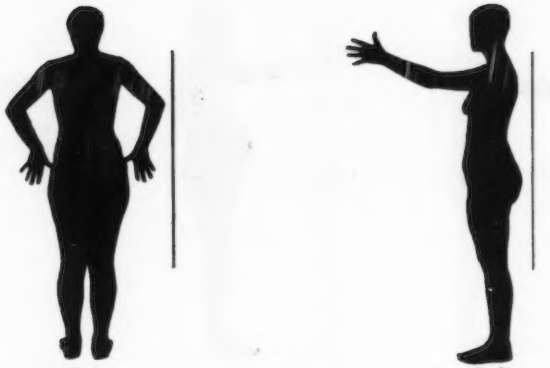


Fig. 1. Photograph of pose C, subject 7.

Fig. 2. Silhouettes of poses A, B, and C, subject 7.



17–A

17–C

1–A

1–C

14–A

14–C

Fig. 3. Silhouettes of poses A and C, subjects 17, 1 and 14.

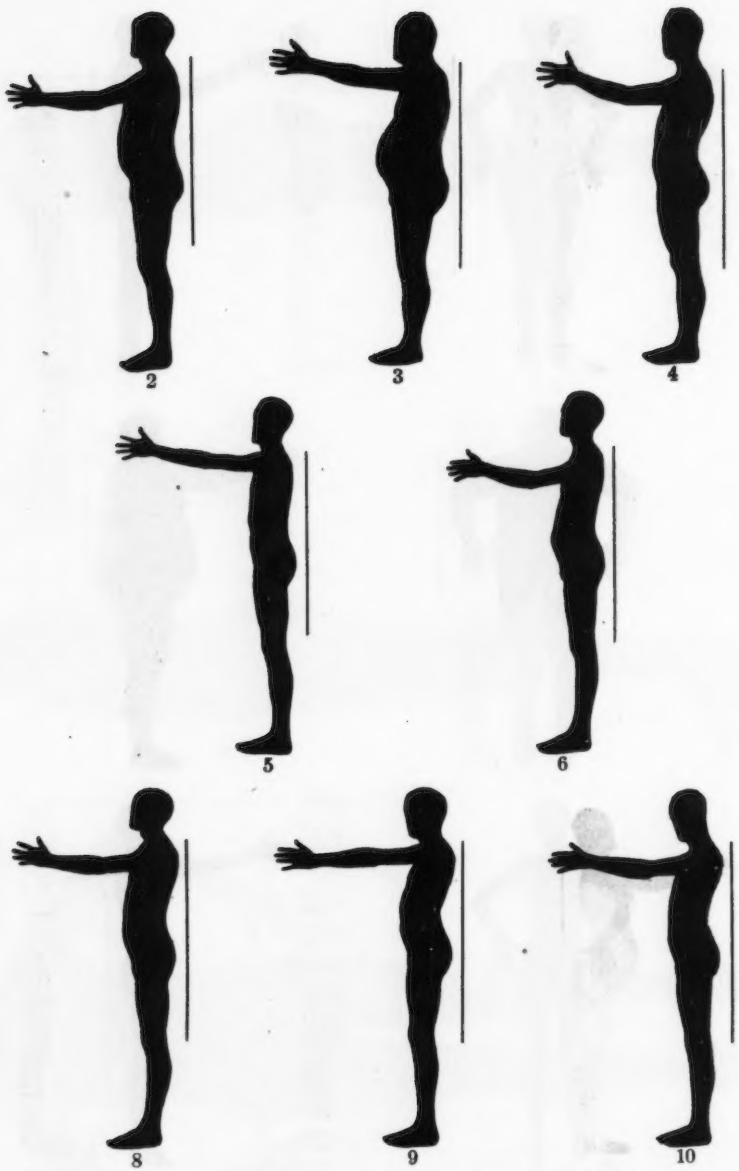


Fig. 4. Silhouettes of pose C for 16 subjects of varying height and weight.

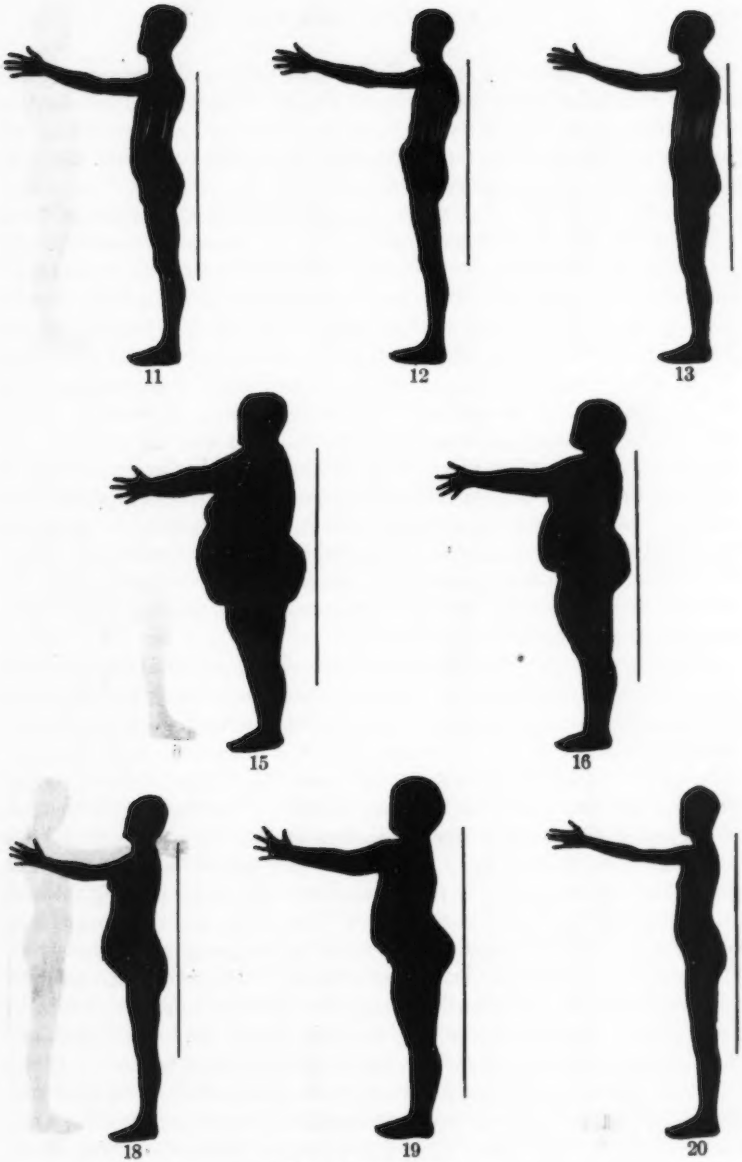


Fig. 4—Continued.

view of the subject with arms akimbo and thumbs resting more or less lightly upon the hips. In planimetering the figure it is important to make allowance for the hair. After very little experience this allowance is readily made and the duplicate measurements with the planimeter show extraordinary accuracy.

Pose B. A profile view of the subject standing at "attention" was selected for pose B. Theoretically this pose shows but one leg. As a matter of fact unless the camera is placed at a very great distance, the other leg will inevitably show, particularly at the feet. Consequently the slight amount of high light on the edges of the leg nearest the camera is distinctly advantageous for indicating the proper outline of the nearer leg for planimetering. In running the planimeter over the photographs of the subject in these poses only the outline of the leg and foot nearest the camera is taken. In adjusting the pose the operator attempts to secure such a position that the leg farthest from the camera will be hidden as much as possible by the leg nearest the camera. With this pose, as with pose A, it is necessary to make allowance for the hair and, with a bearded subject, for the beard. However, even with women, especially when the hair is knotted at the back of the head, no difficulty is experienced in determining the probable contour of the head.

Pose C. Pose B is, however, theoretically unsound inasmuch as it represents the cross section of but one extremity, namely, one leg, while the arms are completely hidden in the shadow of the body. Consequently the subjects were photographed in a third pose, pose C, which differed from pose B in that the left arm was extended in a natural way and the fingers of the hand separated. The original photograph of subject 7 (fig. 1) and the silhouettes of the other subjects in this pose (figs. 2 to 4) show very clearly the position to be adopted. The axis of the arm was directly perpendicular to the plane passing through the limbs and the vertical upright of the body; in other words, the hand should not be brought over into a line opposite the breast bone. With female subjects the arm should be held sufficiently high to show the breast below. In planimetering, the same precautions should be used as with the other poses, i.e., the hair should not be included, and the outline of the leg nearest the camera alone should be used. It was believed that this pose would have special value in indicating some possible relationship between the photographic cross section and the area, particularly in subsequent attempts to secure the surface area of animals in which a cross-section photograph would show the profile of

two extremities. Even with pose C it will be seen that the position is purely empirical since it by no means represents a complete cross-section of the body.

In all three poses the question of the separation of the fingers is of minor technical importance. As a matter of fact, tests have shown that regardless of whether the fingers are close together or are separated, the same results are obtained in planimetering. The separation of the fingers, however, illustrates a little more clearly the possibility of considering the body as a series of short cylinders from which the area of the body may be calculated.

RELATIONSHIPS BETWEEN THE BODY SURFACE AS COMPUTED BY THE DU BOIS FORMULA AND FROM THE PLANIMETERED AREA OF SPECIAL PHOTOGRAPHIC POSES

The observations were made with 20 subjects, of whom six were females. The body weight of the men varied from 144.6 to 32.9 kgm. and of the female subjects from 99.1 to 37.1 kgm. All persons measured were 21 years or over, with the exception of two girls (nos. 16 and 19) who were but $10\frac{1}{2}$ and $6\frac{1}{2}$ years old. The data for the age, nude body weight, and height of the subjects are given in columns *a*, *b*, and *c* of table 1. From these data it will be seen that the subjects varied widely in general configuration of body. Thus, the heaviest individual, with a weight of 144.6 kgm. had a height of but 166.2 cm., while the smallest man was also the shortest. Of the women the heaviest, weighing 99.1 kgm., was likewise the shortest. Thus we have with both the heaviest man and the heaviest woman a typical picture of obesity. Of the normal individuals many were rather thin and several were very tall. A number of the men were members of the laboratory staff and had been most carefully measured on numerous occasions. One of the women (no. 17) was an artist's model, selected for normality of figure. Two of the men (nos. 11 and 12) and two of the women (nos. 18 and 20) were diabetics and were somewhat emaciated.

I wish here to express my indebtedness to Dr. E. A. Locke for his kindness in putting me in communication with the obese subject 1, to Dr. F. B. Talbot and his assistant, Dr. J. M. Lee, for their help in securing the coöperation of subjects 16 and 19, and to Dr. E. P. Joslin for the diabetic patients. I am especially indebted to Dr. J. H. Means not only for securing the assistance of the obese subject 15 but for many helpful suggestions. Without the coöperation of the subjects (5) little

could have been accomplished and to all I desire to express my thanks, especially to my intimate associates who volunteered and also assisted in the numerous measurements.

Cross section photographs were made of all of the subjects in the poses A, B, and C, and by means of the planimeter and the measurement of the photographed meter stick, the total area shown in the photograph, or the true area of the body in that particular section, was computed. The method of computation was as follows:

Owing to the size of the paper used, it was generally necessary to divide the body into two approximately equal parts by a line passing through the hips. The upper and lower sections were planimetered separately and the sum of these two areas, which on the instrument we used was expressed in square inches, was multiplied by the factor 6.45 for conversion to square centimeters. The actual length of the reduced meter stick was measured in millimeters (generally from 104 to 114 mm.) and the true area obtained by a simple proportion.

Thus, using subject 7 for an illustration, the photograph of pose C, reproduced in figure 1, gave for the lower part of the body 3.11 sq. in., and for the upper part 3.40 sq. in., with a total of 6.51 sq. in. This, multiplied by the factor 6.45, gave a total of 42.0 sq. cm., or, as our result is to be expressed in square meters, 0.00420 sq. m. The meter scale as reduced in the photograph measured 108.25 mm. or 0.10825 m. The proportion used, therefore, was as follows:

$$l^2 : 1^2 : : a : x,$$

l representing the length of the reduced meter scale (0.10825 m.), a the area of the photograph expressed in square meters (0.00420 sq. m.), and x the surface area of the section of the body shown in pose C (0.358 sq. m.). The general equation would therefore be:

$$x = \frac{a}{l^2}$$

The calculated areas based upon the various photographic measurements for poses A, B and C, expressed in square meters for each of the subjects, are given in columns d, e, and f of table 1. As would be expected, the values for pose A are the largest and the values for pose C are larger than those for pose B owing to the additional area of the extended arm and hand.

Since the entire investigation is based upon an assumption of the fundamental accuracy of the Du Bois formula, each of these subjects was

most carefully measured, usually by two, frequently by three individuals, and after numerous personal conferences with Dr. E. F. Du Bois. I would like at this point to express my warmest appreciation of the assistance and great number of suggestions that Dr. Du Bois has given me, both by personally instructing members of the Nutrition Laboratory staff in the technique of measuring and by assisting in clarifying several obscure points in obtaining accurate measurements by this formula. The area of each individual[1] as computed from the Du Bois measurements is given in column *i* of the table. These areas represent the base line to which all the values subsequently computed are referred. The values computed by the Meeh formula are given for comparison in column *j*.

RELATIONSHIPS OF AREA OF PHOTOGRAPHIC POSES TO TRUE BODY SURFACE

When the photographs of the subjects in the three poses A, B, and C were examined certain astonishing relationships were almost immediately noted. Neither pose A nor pose B take into consideration the area of the ends of the body, i.e., the bottom of the feet and the top of the head and shoulders. Furthermore, it is obvious that since the body is not as flat as a leaf, the surface of the body will be larger than twice the area of the cross section shown in A, but how much more remains to be determined. If we consider the body as made up of a series of short cylinders without ends, we might express the relationship between the photographic surface A and the true surface by the formula πA. On the other hand if we take twice the area in pose A and add to it twice the area of the profile view (pose B) we should assume that the body was a rectangle in shape; we should still have no measure of the area of the ends of the body. As a result of these pre-

[1] It is especially important with obese subjects in taking the measurements L and W of the Du Bois formula to make the measurements between perpendiculars. Thus, the distance in the length between the suprasternal notch to the upper border of the pubes with a fat person, if measured by a tape laid directly on the skin, will represent a distinctly curved distance, and hence the length will be too long. Similarly the length W, which is from the upper border of the pubes to the lower border of the patella, should likewise be measured between perpendiculars. Owing to difficulties in locating the superior border of the great trochanter with fat people the value W has been used instead of the value O. Dr. Du Bois has kindly supplied a constant where W is used, this constant being 0.552. See Du Bois and Du Bois; Arch. Intern. Med., 1916, xvii, 863.

liminary considerations the following formulas were selected, first, πA, and second, $2A + 2B$. It will be seen that πA represents the surface of a cylinder of the area of the cross section A, without taking the ends into consideration, while $2A + 2B$ indicates a rectangular formation, also without including the ends.

The values obtained by using the first formula (πA) are given in column *g* of table 1. A comparison of these values with those obtained with the Du Bois formula as given in column *i* shows somewhat lower values for those computed from the formula πA. On the other hand it will be noted that these values are very much nearer the Du Bois values than are those calculated by the Meeh formula and given in column *j*, these latter values being invariably somewhat higher. The values as computed by the second formula ($2A + 2B$) and given in column *h* of the table show usually a striking uniformity with those obtained with the first formula, πA, and likewise a closer approximation to the Du Bois values than the Meeh values. This uniformity in the values as computed by the formulas πA and $2A + 2B$ appears throughout practically the entire series, the greatest discrepancies being shown with the two obese individuals, nos. 1 and 15. Numerous other interesting relationships were established between the various cross-section photographs, their perimeters, the Du Bois formula, and the area computed from the Meeh formula.

As an inspection of the values obtained by means of the formula πA shows that the results were invariably somewhat lower than those found with the Du Bois linear formula, it is obvious that a factor somewhat larger than π is essential for computing the true surface area from the cross-section photographs of the subjects in pose A. To obtain this factor the values found by the Du Bois formula were divided by the values given in column *d* for the areas of the photographs of the pose A. The results of this computation are given in column *k* and are in all cases higher than π, ranging from 3.21 to 3.89, with an average value for the 20 subjects of 3.48. Using this constant alone, therefore, we may compute the body surfaces of this heterogeneous group of individuals, with very wide variations in body contour, weight, and height, and obtain results varying only from + 11.6 to −7.7 per cent from the true body surface, a majority of the values lying within ± 4 per cent.

Using the area of the photograph in pose B as the basis, and dividing the total areas as computed by the Du Bois formula by the areas of the pose B photographs as found with the planimeter, we obtained the

TABLE 1

Measurements of the body surface of men and women by various formulas

SUBJECT NO.	AGE	NUDE BODY WEIGHT	HEIGHT	AREA SHOWN IN VARIOUS POSES			TOTAL BODY SURFACE				DU BOIS AREA DIVIDED BY			TOTAL BODY SURFACE 5.02 C	DIFFERENCE BETWEEN DU BOIS AND PHOTOGRAPHIC METHODS
				A Back	B Side	C Side, with arm extended	Photographic measurements		Du Bois formula	Meeh formula $12.312 l^{\frac{1}{2}} W^{\frac{1}{2}}$	A $i \div d$	B $i \div e$	C $i \div f$		
							πA	$\frac{1}{2}A + 2B$							
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)	(o)
	years	*kilos.*	*cms.*	*sq. m.*	*sq. m.*	*sq. m.*	*sq. m.*	*sq. m.*	*sq. m.*	*sq. m.*				*sq. m.*	*per cent*
Males															
1	30	144.6	166.2	0.692	0.467	0.536	2.17	2.32	2.69	3.39	3.89	5.76	5.02	2.69	0
2	45	87.1	182.2	0.618	0.360	0.422	1.94	1.96	2.10	2.42	3.40	5.83	4.98	2.12	+1
3	39	84.8	166.3	0.566	0.335	0.399	1.78	1.80	2.04	2.38	3.60	6.09	5.11	2.00	−2
4	23	70.4	170.8	0.570	0.308	0.381	1.79	1.76	1.91	2.10	3.35	6.20	5.01	1.91	0
5	21	69.0	185.4	0.573	0.321	0.385	1.80	1.79	1.95	2.07	3.40	6.07	5.06	1.93	−1
6	48	60.4	179.7	0.522	0.285	0.343	1.64	1.61	1.74	1.90	3.33	6.11	5.07	1.72	−1
7	23	59.8	172.9	0.518	0.298	0.358	1.63	1.63	1.74	1.88	3.36	5.84	4.86	1.80	+3
8	36	59.1	176.1	0.509	0.295	0.338	1.60	1.61	1.71	1.87	3.36	5.80	5.06	1.70	−1
9	27	57.5	173.5	0.490	0.282	0.341	1.54	1.54	1.73	1.83	3.53	6.13	5.07	1.71	−1
10	26	57.1	174.3	0.521	0.283	0.332	1.64	1.61	1.67	1.83	3.21	5.90	5.03	1.67	0
11	24	53.6	167.5	0.487	0.267	0.329	1.53	1.51	1.59	1.75	3.26	5.96	4.83	1.65	+4
12	55	49.5	166.4	0.464	0.263	0.320	1.46	1.45	1.63	1.66	3.51	6.20	5.09	1.61	−1
13	37	48.0	164.8	0.434	0.260	0.307	1.36	1.39	1.47	1.63	3.39	5.65	4.79	1.54	+5
14	24	32.9	148.1	0.349	0.202	0.239	1.10	1.10	1.19	1.26	3.41	5.89	4.98	1.20	+1
Females															
15	50	99.1	144.7	0.540	0.363	0.421	1.70	1.81	2.09	2.64	3.87	5.76	4.96	2.11	+1
16	$10\frac{1}{2}$	86.5	144.3	0.533	0.332	0.383	1.67	1.73	2.00	2.41	3.75	6.02	5.22	1.92	−4
17	24	56.6	159.6	0.481	0.264	0.321	1.51	1.49	1.65	1.81	3.43	6.25	5.14	1.61	−2
18	56	49.1	156.6	0.413	0.253	0.299	1.30	1.33	1.53	1.65	3.70	6.05	5.12	1.50	−2
19	$6\frac{1}{2}$	44.3	126.8	0.361	0.228	0.259	1.13	1.18	1.29	1.54	3.57	5.66	4.98	1.30	+1
20	35	37.1	151.7	0.395	0.220	0.263	1.24	1.23	1.31	1.37	3.32	5.95	4.98	1.32	+1
Average...	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.48	5.97	5.02		

constants recorded in column *l*. As would naturally be expected in view of the smaller area shown in pose B, we find that the constants are somewhat higher than those for pose A, ranging from 5.65 for subject 13 to 6.25 for subject 17 (the artist's model), with an average value of 5.97. The variation from the average is even less for pose B than for pose A, being at the most approximately ± 5 per cent. With either series of constants, but especially with the second, values much nearer to the true body surface may thus be obtained than with the Meeh formula.

When we compute the values from the areas obtained from the planimetered surface of pose C, we find even more concordant results (see column *m* of table 1). These values range from 4.79 with subject 13 to 5.22 with subject 16, the obese girl; the average value is 5.02. The extraordinary constancy exhibited by these values is certainly most difficult to explain. It may be shown more clearly if we use this constant of 5.02 to compute the surface area from the planimetered area of pose C for each subject and compare the results (5.02 C.) with the values obtained from the Du Bois measurements and the Du Bois formula. This comparison is made in columns *n* and *o* of table 1 in which we find that of the 20 subjects three show values in practically perfect agreement with the true area as determined by the Du Bois formula, ten vary only 1 per cent, three 2 per cent, one 3 per cent, two 4 per cent, and one 5 per cent. We see, therefore, that the maximum error in using this constant is 5 per cent; the average error with this factor is ± 1.6 per cent. In view of the fact that the data for the subjects given in table 1 are arranged in order of the decreasing body weight and hence approximately in order of the decreasing body surface, it is astonishing to note the distribution of the agreement of the computed values with the true body surface. The largest man and the smallest man both show nearly perfect agreement; the same happens to hold true for the largest and smallest of the relatively few female subjects. It will be seen from these computed values that a singularly constant relationship exists between the true body surface as measured by the Du Bois formula and the area of a silhouette photograph of a side view of the body with the left arm extended. In addition to supplying an accurate basis for body surface measurements the silhouette gives ocular evidence of the general configuration of the body, a factor of especial value in observations on obese, emaciated, and abnormal individuals.

SUMMARY

Primarily with the object of securing measurements for a subject who had undergone a 31-day fast, a photographic method was developed to establish the relationship between the area of the body computed from certain definite photographic poses (particularly a side view with arm extended) and that actually measured by the Du Bois linear formula. A method for securing accurate measurements of the body area resulted. Comparisons between the photographed areas and the body surface as computed from the Du Bois linear formula show, even with the most diverse configurations of body, a constancy rarely observed in anatomical measurements or in computed ratios based upon such measurements.

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THE RELATIONSHIP BETWEEN BODY SURFACE AND HEAT PRODUCTION ESPECIALLY DURING PROLONGED FASTING

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One of the earliest outcomes of an attempt to explain the relatively higher food demands of small animals of a given species as compared with those of larger animals of the same species was the formulation by Bergmann (1) of the generalization that the heat production of a body is proportional to its surface. No direct experimental evidence bearing upon this particular point appeared until 40 years later, when Rubner (2) in his classical research on animal calorimetry, maintained that the heat lost from living organisms is essentially constant per unit of body surface. While this constancy in relationship between body surface and heat production is the dominant note in Rubner's writings, it is well to remember that in several instances he writes as if a causal relationship between body surface and heat production was by no means thoroughly established. On the other hand, Richet (3) in his writings published about the same time, stated without qualification, that the heat production of a warm-blooded organism is determined by its body surface.

The apparent establishing by Rubner of a simple mathematical relationship between body surface and total metabolism became naturally the subject of much discussion. The range in the animal kingdom over which this supposed law extended was indeed astonishing, the interesting computations of E. Voit (4) appearing to show that animals ranging in size from a 2-kilogram fowl to a 441-kilogram horse have essentially the same heat production per square meter of body surface, namely, 970 calories per 24 hours.

It is true that marked variations from the general law were soon noted but attempts were made to explain these discrepancies by the nature of the integument, the density of the fur and hair coverings,

and variations in the amount of body surface exposed; in this connection we frequently find in the text of the earlier writers such statements as the following from Rubner: "Wärmeabgebende Fläche und Hautfläche sind zwei sehr verschiedene Dinge." (5)

The members of the Zuntz school in Berlin were extremely cautious about accepting the general thesis as to the relation between body surface and metabolism. As possibly representative of this we may quote the following expression from Magnus-Levy and Ernst Falk (6).

> Welche inneren Ursachen diesem Gesetze zu Grunde liegen, ob die behauptete Abhängigkeit eine directe oder indirecte ist, haben wir hier nicht zu erörtern; wir wollen bloss prüfen, ob unsere Zahlen mit dieser Annahme übereinstimmen oder nicht. Eine ausführliche kritische und theoretische Erörterung der obigen Lehre findet sich in v. Hoesslin's (7) gedankenreichem Aufsatze: "Ueber die Ursache der scheinbaren Abhängigkeit des Umsatzes von der Grösse der Körperoberfläche."

Von Hoesslin's paper attracted considerable attention but the general view that the body surface determines the metabolism has predominated essentially since that time. The relationship between the body surface and Newton's law of cooling was perhaps more emphasized by the members of the French school than by the Germans; yet when the influence of environmental temperature was studied, difficulties were immediately encountered and we find certain interesting considerations brought out by d'Arsonval (8) in an article giving a summary of his numerous investigations covering a period of six years. In discussing the fact that certain animals show abnormal relationships between the environmental temperature and their body temperature, he introduces the following significant sentence:

> Cela tient évidemment à ce que la surface rayonnante *physiologique* de l'animal n'est pas constante comme sa surface *physique*. Aux basses températures, le phénomène se complique d'une constriction vasculaire périphérique, qui restreint considérablement le pouvoir rayonnant de l'animal à égalité de surface *physique*. Cela montre que la connaissance de la surface géométrique d'un animal est insuffisante pourqu'on en puisse déduire la perte par rayonnement; il faut encore tenir compte de l'état de la circulation périphérique.

Finally, a hint of the extremes to which too strict an adherence to the law of body surface may lead us is given in Pütter's contention (9) that we should take into consideration the "active" surface, i.e., the cell surfaces of the various organs of the body, Pütter maintaining that the energy consumption is proportional not to the body surface but to the area of the lung surface.

In this connection it should certainly be pointed out that a not inconsiderable proportion of the total heat output of the day is given off from the lungs through the warming of the air and through the vaporization of water. No system of body surface measurement with which we are thus far familiar takes into consideration this rather important factor. It has been computed as a result of a large number of experiments with human subjects at rest, either with or without food,that from 11 to 12 per cent of the total heat output of the day is required to heat the inspired air and to vaporize the water in the lungs(10). It has likewise been computed that only about 75 per cent of the total heat is given off by radiation and conduction, and approximately 12 per cent by water vaporized from the skin. Certainly the 11 to 12 per cent of the total heat produced which is required to warm the inspired air and to vaporize the water in the lungs—two factors that have no relation whatsoever to body surface—is not, so far as we are aware, considered in any discussion of body surface in its relation to basal metabolism.

It is thus clear that there has been at no time a fixed interpretation of the relationship between body surface and heat production for even its most ardent advocates have at times called attention to noticeable abnormalities. Unfortunately many modern writers are not so conservative in their expressions as to the cause for this relationship between body surface and heat production as were the earlier writers. In his deservedly oft-cited contribution on respiration to Schaefer's Physiology Pembrey says (11):

> Now small mammals and birds have a temperature equal to or even higher than that of large animals of the same classes; and, on account of the relatively greater surface which they expose for the loss of heat, they must have a relatively far greater production of heat than the large animals, for there is generally no marked difference in the protective coat of fur or feathers.

Although Lefèvre specifically states that the application of the law of Newton to living animals is illusory (12) nevertheless in his discussion of the production of heat per unit of surface the following statement appears:

> La production chez l'homéotherme est *en équation* avec la perte calorique. Or, à pouvoir émissif égal, la déperdition est évidemment proportionnelle à la surface rayonnante. La production calorique (c'est-à-dire, chez l'organisme en équilibre et au repos, le besoin d'énergie) est donc proportionnelle à l'étendue de la surface totale du corps (13).

Furthermore, Professor H. P. Armsby, undoubtedly the foremost writer in America on animal nutrition, states:

> The results which we have been considering show that in general the emission constant, i.e., the rate of heat emission per unit of surface, is substantially the same in small and large animals, and that the greater loss of heat in the former case is met by an increased production. In this aspect the effect is simply an extension of the influence of falling temperature, the increased demand for heat being met by an increased supply, so that the extent of surface appears as the determining factor of the amount of metabolism (14).

Even in current (1915) literature we find such statements as the following:

> Rubner's law, to quote from Lusk, is that "the metabolism is proportional to the superficial area of an animal. In other words, the metabolism varies as the amount of heat loss at the surface, and its variance in accordance with this law is necessary for the maintenance of a constant temperature" (15).

In a popular textbook on nutrition we also find:

> Since the body loses heat in proportion to the extension of its surface, it is not strange that this is the determining factor for the metabolism (16).

Between 1885 and 1915 practically no direct comparison of measured body surface and metabolism was made. The technique for body surface measurements is very complicated and investigators as a whole have accepted the measurements of Meeh (17) in which the body surface is equal to the two-thirds power of the weight multiplied by a constant which, for man, is 12.312. The constants for animals, which figure so prominently in E. Voit's (18) remarkable calculations apparently demonstrating the universality of the law, have rested undisturbed in the textbooks for many years, together with that for human organisms, and have rarely been questioned. On the other hand they have frequently had to bear the brunt of the explanation for seeming discrepancies in the application of the law, a good illustration of this being the attempted explanation of the difference in metabolism between atrophic and normal infants on the ground that, with atrophics, there is a disproportion between weight and surface (19).

The recent observations of Moulton (20) are also not without significance in connection with the possibility of errors in the factors for obtaining the true body surface.

Recognizing the probable errors in the values given by the Meeh formula and stimulated by a desire to correlate basal metabolism and

body surface more exactly, D. and E. F. Du Bois (21) have developed an exact linear formula which is destined to revolutionize completely our conception of body surface. With this formula it may be said that, given certain body measurements, the body surface of any human individual may be calculated with an unusual degree of accuracy; furthermore, a photographic method for measuring the body surface which has recently been developed in the Nutrition Laboratory (22) fully confirms the fundamentality of the formula. In the hands of the Du Boises and those trained under them, the measurements are certainly made with accuracy and expedition. Unfortunately they do not give one any conception of the general configuration of the body measured, even when accompanied by records of weight and height, the two factors most commonly reported in metabolism studies. That the Du Boises have succeeded in substituting for the admittedly uncertain Meeh formula a method of measurement having a degree of accuracy rare in anatomical measurements is a cause for congratulation. Their contribution to body surface measurement awakens new interest in the relationship between body surface and metabolism.

It is the consensus of opinion of physiologists that the preferable basis of measurement for the metabolism of human organisms is not per kilogram of body weight but per square meter of body surface. The evidence secured in this laboratory leads us to believe that this is due not to the loss of heat from the body surface but to the fact that the body surface is a better index of the general law of growth, in full conformity with the principle laid down by v. Hoesslin (23).

The specific purpose of determining the body surface, therefore, is that it may be used for computing the heat production per unit of surface as a basis for comparison between various individuals. In this method of comparison a value for basal metabolism which is derived from the average results obtained with a large number of persons has been suggested as the "normal" or "standard" value to be applied to each individual case. It will be seen that a prerequisite for such a method is an exact knowledge of the surface area. This method also assumes a universality in the application of the law of surface area.

The belief in this universal applicability of the so-called law of surface area is so widespread that we find a strong tendency among physiologists to apply the law upon all occasions. If there is a law of surface area, i.e., if the heat production of the living animal is proportional to its surface area, we should examine the results of metabolism studies closely to find out to what extent this law may be applied and more

especially to find out if the metabolism of human individuals maintains a strict proportionality to body surface.

Several researches carried out in this laboratory will, it is believed, contribute extensively to our knowledge of the relationship between body surface and metabolism. In accumulating experimental evidence on the normal metabolism of men and women at the Nutrition Laboratory, we have strongly contended against the generally accepted view of a causal relationship between body surface and heat production, and an attempt was made in a series of papers recently published to clarify the situation by an analysis of the basal metabolism of 89 men and 68 women (24). The primary object of this series of papers was to discuss the probable factors controlling basal metabolism and it was frankly stated that the loss of heat from the body surface could not be considered as the determining factor. Unfortunately these papers have been interpreted, in part at least, as discouraging any efforts to secure accurate measurements of body surface. This was far from the intention of the writers. The accurate measurement of body surface is an important physiological problem, solved in a highly creditable manner by E. F. and D. Du Bois. The use of body surface as an index of basal metabolism is a wholly different problem. That there is a relationship between the body surface and metabolism no one can deny. That this relationship is causal or sufficiently exact to be designated as a physiological law may be fairly questioned.

As a result of the interpretation of the values obtained with normal men and women, certain factors, such as sex, age, and athletic training, were demonstrated as affecting the basal metabolism, even on the basis of per square meter of body surface, thus showing that there are distinct exceptions to the so-called body surface law. It is of interest to note that the existence of three of these factors has been admitted by other writers.

In an experiment carried out upon a dog which was confined to the laboratory for several months and which did not lose weight, it was proved (25) that the metabolism decreased very considerably (19 per cent). When the dog was again allowed country life, her metabolism returned to essentially its original value but the body weight was unchanged. Here evidently there was a constancy in the body surface but a noticeable difference in the heat production per square meter of body surface. We therefore have still another demonstrated factor affecting basal metabolism and vitiating the universal applicability of the body surface law.

As an index of the caution exhibited by Rubner in presenting this law we have the following interesting statement:

> Das Oberflächengesetz gilt unter allen physiologischen Lebensbedingungen, zu seinem Beweise ist aber sinngemässe Voraussetzung, dass nur Organismen mit gleichartigen physiologischen Leistungen, was Ernährung, klimatische Einflüsse, Temperament und Arbeitsleistung trifft, verglichen werden (26).

As a matter of fact this particular quotation has been cited in adverse criticism of conclusions from this laboratory regarding the comparison of the basal metabolism of normal and atrophic infants. In other words, judged upon this basis, the body surface law could not be expected to hold with individuals under different physiological conditions.

At the time that our analysis of normal data was made, our only knowledge of body surface was that supplied by the antiquated formula of Meeh; in view of the development of later and more exact methods of measurement, a re-analysis of the large mass of metabolism measurements is certainly necessary. If correction is made for the gross errors in the Meeh formula it is conceivable that the discrepancy between heat production and body surface may be reduced or, indeed, wholly disappear. But the revision of the earlier formulas throws discredit upon the previous selection of constants and if the errors in the Meeh formula are so large as those pointed out by the Du Boises, one may also reasonably question the formulas for lower animals; it is thus probable that the final computations of E. Voit may likewise need a radical revision.

It should be noted, however, that in our analysis of normal material and in the comparisons of diabetics and normal subjects, vegetarians and non-vegetarians, athletes and non-athletes, another method of comparison was used which is entirely independent of surface area measurements. This may be designated as the "group system" of comparison, by which the metabolism of groups of persons of like height and weight are compared on the fundamental assumption that individuals of like height and weight have the same general growth relations. Thus far no method of measurement that has been proposed affects the reliability of such a procedure and no existing experimental evidence diminishes in any way one's confidence in this form of comparison.

With the new-born infants that we have studied in this laboratory, many of the complex factors, such as age, variations in muscular activity, changes in diet, etc., which apply to adults, also the variations in growth,

are eliminated; hence it seemed desirable to study the influence of variations in the body surface upon the metabolism of these infants. Using the Lissauer formula, in which the constant differs somewhat from that in the Meeh formula, we found no obvious relationship between normal and atrophic infants and no approximation to a physiological "law" was observed until infants between one day and six days old were studied. At that age it was found that if the heat production per square meter of body surface per 24 hours, as computed by the Lissauer formula, was divided by the length of the infant a relatively constant factor of 12.65 calories was obtained.

We still believe that the method of group comparison used for diabetics, athletes, vegetarians, and men and women is fundamentally sound. For a true knowledge of the heat production in various diseases there is no reason why the group system of comparison should not be employed as but little definite information may be expected until a considerable number of cases of the particular disease to be studied is obtained. For a study of the metabolism during prolonged fasting, however, it is impracticable to obtain a sufficient number of cases to permit the use of this method; consequently a 31-day fasting experiment made in this laboratory has a particular interest in this connection (27). The differences in the metabolism shown in this fasting experiment during complete muscular repose were so striking and the bearing of these variations upon the law of surface area is so great that an accurate determination of the body surface of the man as the fast progressed is essential for comparative purposes since it is obviously impossible to make a group comparison for each stage of the fast.

While from time to time constancy or equality in the condition of nutrition has been emphasized as a prerequisite when the law of surface area is to be applied, nevertheless this has by no means been generally considered as a necessity, and we find current practice tends to accept the universality of this law irrespective of whether the individual is poorly or well nourished. Information with regard to the metabolism of individuals that are well or poorly nourished is, for the most part, obtained by observations on different individuals. But during prolonged fasting we may observe in the same individual changes in the plane of nutrition fully comparable to those roughly characterized as poorly or well nourished. It is thus seen that during prolonged fasting simultaneous measurements of the body surface and the basal metabolism of the subject have an unusual value.

A study of the relationships of body weight, body surface, and basal

metabolism during fasting is all the more important when it is remembered that it is commonly believed that the fasting animal rapidly adjusts itself to the minimum metabolism. The results of earlier experiments on the dog, the cock, and the guinea pig (28) indicate that per kilogram of body weight the fasting metabolism is constant. With the fasting man studied in this laboratory the metabolism per kilogram of body weight did not indicate constant values, although the measurements were made under identical conditions of muscular repose. This lack of constancy on the basis of per kilogram of body weight led to the computation of the metabolism on the basis of per square meter of body surface, the body surface being determined by means of the Meeh formula. But these values likewise showed no constancy.

During so long a fast as this there was naturally a marked change in the body weight and a change in the configuration of the body. According to tradition any disturbances in the relationship between body surface and heat production could be attributed to a possible disturbance in the relationship between body weight and the computed body surface. Although in the discussion of the results due consideration was given to the possibility of such a disturbance in the relationship between body weight and body surface as the fast progressed, yet, using Meeh's formula for computing the body surface—the only one available at that time—we found a large loss in the heat production per square meter of body s irface during the progress of the fast, with variations towards the end which indicate a distinct change in the stimulus to the metabolic activity of the whole body. While it is highly improbable that an error in the Meeh formula would make it so inapplicable to the same individual at the beginning and end of a 31-day fast as to invalidate completely the differences in the metabolism found on different days, nevertheless, as was pointed out in the discussion of the subject, (29) the metabolism of this man per square meter of body surface as measured by the Meeh formula is undoubtedly erroneous so far as its absolute value is concerned and, in all probability, is somewhat erroneous so far as the differences are concerned.

In view of the variations in the metabolism per square meter of body surface shown by this fasting subject when computed on the Meeh basis, it seems all the more important to obtain accurate measurements of the body surface of this man by the new method of the Du Boises. The anthropometric measurements made of the subject at the time of the fast (30) do not give the data required by the Du Bois linear

formula. It has been possible, however, by employing a photographic method recently developed in the Nutrition Laboratory (31), to recompute the body surface from a series of anatomical photographs taken of the fasting man at intervals during the study (32). The body area shown in the photographs was measured with an accurate planimeter. Similar measurements were made of photographs of a number of normal individuals whose body area had been determined by the Du Bois formula. With the results obtained from these photographs and from the planimetered photographs of the fasting subject, the body surface of the fasting man could be obtained by a simple proportion.[1] We were thus able by an indirect method to revise the values for the body surface at different stages of the fast.

Since the metabolism of the subject of this 31-day fasting experiment has a special significance in its bearing upon the legality of comparing directly the basal metabolism of a general group of normal, robust individuals with that of a group of individuals emaciated by disease, the heat production per square meter of body surface of our subject during his 31-day fast has been recomputed on the basis of these new values for body surface and the results obtained are here discussed.

[1] It should be stated here that the technique and poses used in taking the photographs of the fasting man were not those finally decided upon as the most satisfactory for the photographic method. Accordingly the photographs of normal subjects used for comparison were those taken in the earlier stages of the development of the photographic method in which the poses were similar to those used with the fasting man. This fact is specially emphasized, since photographing the body against a white sheet, as described under "Technique" in the description of the method published in the accompanying article (Benedict, this Journal, 1916, xli, 275), was a later development of the photographic method of securing a measure of the body surface.

As this manuscript goes to press the latest contribution of the Du Boises to the technique for the estimation of body surface has appeared in the form of a most ingenious height-weight chart (Amer. Journ. Med. Sci., 1916, cli, 781), based fundamentally upon their linear formula but in the final computations considering simply height and weight. The height-weight chart thus gives tacit assent to the validity of our earlier assumption that individuals of like weight and like height have the same surface area and are hence perfectly comparable. A comparison of the measured surface obtained on our subjects with the values found in the height-weight chart shows that for individuals of average height and weight and, indeed, for thin individuals the agreement is very satisfactory. Unfortunately, for the obese this is not invariably the case. It should be stated, however, that the Du Boises have not projected their curve to include distinctly obese individuals. It is of especial interest in this connection to note that the areas on the Du Bois height-weight chart for the subject of the prolonged fast, A. L., agree perfectly with those found by the photographic method.

HEAT PRODUCTION DURING PROLONGED FASTING

In this study of a fasting man absolutely no food was taken by the subject for 31 days and only about 900 cc. of distilled water was given him daily. The heat production during the night was measured directly with the bed calorimeter for each of the 31 nights. It was likewise computed indirectly from the carbon-dioxide excretion and oxygen consumption during the same period. It was immediately observed that as the fast progressed there was a very noticeable difference in the heat production from night to night. This would naturally be expected, since the individual grew smaller each day, the initial weight being not far from 60 kgm., while the final weight was about 47.5 kgm. On the general assumption that a small animal will give off less heat than a large animal, a quantitative decrease in metabolism would thus be expected. The results of the heat measurements of this individual have been considered in detail elsewhere and reference must be made to the original publication (33) for the methods of calculation and for the discussion of the heat production per kilogram of body weight in which an attempt was made to reduce the observations of each night to a common standard. Only the metabolism per square meter of body surface is considered in this paper.

The subject of our 31-day fasting experiment may be considered to have been at the beginning of the fast a normal individual, i.e., a person in presumably good health; he would therefore be perfectly comparable to those individuals without obvious pathological lesion who have also been studied in this laboratory. During the progress of the fast he remained (so far as ocular evidence is concerned) for the most part physiologically normal. The strength tests made with the hand dynamometer during the fast showed practically no change with the right hand and but a slight fall wth the left hand, although there was an almost immediate evidence of fatigue in the first two or three days of the fast. While there was naturally a certain amount of weakness observable in the last few days, an idea of the strength of this man may be obtained from the fact that after having been without food for 31 days he spoke extemporaneously before a body of physicians for approximately three-quarters of an hour, standing during the whole period and vigorously gesticulating. Later in the day he actually sang and danced at times in anticipation of the closing of his long fast. It is thus clear that we have here to do not with a fasting man who is in the last stage of emaciation and in a moribund condition but with

an individual who, judged from ocular evidence, would appear not at all unlike the normally emaciated type of individual.

Furthermore, the body temperature during the fast did not materially alter. Indeed, his average body temperature in the bed calorimeter experiment on the night of the last day of the fast was but 0.3°C. below that of the night of the second day, a difference in body temperature that is wholly comparable with that observed by Lusk with his dog during the pre-cage confinement, just after prolonged confinement, and after the dog's return from the country. In other words, we have here no marked disturbance of the body temperature.

Although this man's pulse was distinctly lower at the end of the fast than at the beginning, it will be seen that he underwent the 31-day fast without a great loss of muscular strength or a material alteration of the body temperature. In spite of the absence of these disturbing factors the metabolism when computed on the basis of per kilogram of body weight showed a decided loss as the fast progressed. There was also a loss in metabolism per square meter of body surface; this is shown by the data in table 1, in which are given the nude body weight, the body surface as computed by the Meeh formula and from the measurements of the anatomical photographs, and the heat production per square meter of body surface per 24 hours as based upon the observations with the bed calorimeter during the night. In computing the heat production per square meter of body surface per 24 hours, use has been made of the body surface as computed from both the Meeh formula and the photographic measurements.

It will be seen from the figures given in table 1 that, using as a standard the body surface values obtained with the photographic method, the body surface as computed from the Meeh formula is invariably too large and consequently the heat production per square meter computed from this measure of the body surface is too small. Disregarding the last food day prior to the fast, we find that the heat production per square meter per 24 hours as given in the last column of the table ranges from 927 calories on the third day of the fast to as low as 664 calories on the twenty-first day of the fast, representing a decrease in the heat production per square meter of body surface of 28 per cent. Thereafter there was a distinct tendency for the heat production to increase.

In the absence of any marked change in body temperature the difficulty of considering the loss of heat from the surface of the body as the determining factor in the metabolism of this fasting man is very

TABLE 1

Heat produced by fasting subject during experiments in bed calorimeter at night
(Fasting period 31 days from April 14 to May 15, 1912)

DATE	DAY OF FAST	BODY WEIGHT WITHOUT CLOTHING	BODY SURFACE		HEAT PRODUCED PER SQ. METER PER 24 HOURS	
			By Meeh formula $12.312\sqrt[3]{W^2}$	Computed from photographic measurements	Meeh formula	Photographic method
1912		*kilos*	*sq. meters*	*sq. meters*	*cals*	*cals*
April 13–14		60.87	1.91	1.71*	858	958
14–15	1st	59.86	1.88	1.70	817	904
15–16	2nd	58.91	1.86	1.68	830	918
16–17	3rd	58.01	1.84	1.66*	836	927
17–18	4th	57.22	1.83	1.66	827	912
18–19	5th	56.53	1.81	1.66	764	833
19–20	6th	56.01	1.80	1.65	774	845
20–21	7th	55.60	1.79	1.65	760	825
21–22	8th	55.18	1.78	1.65	790	852
22–23	9th	54.74	1.77	1.65	720	772
23–24	10th	54.25	1.77	1.65*	725	778
24–25	11th	53.94	1.76	1.64	715	767
25–26	12th	53.64	1.75	1.64	712	760
26–27	13th	53.48	1.75	1.63	709	761
27–28	14th	53.22	1.74	1.62	698	749
28–29	15th	52.92	1.74	1.62	649	698
29–30	16th	52.40	1.73	1.61	639	687
April 30–May 1	17th	51.91	1.71	1.60*	642	686
May 1– 2	18th	51.57	1.71	1.60	653	698
2– 3	19th	51.21	1.70	1.60	676	719
3– 4	20th	50.97	1.69	1.60	666	704
4– 5	21st	50.60	1.69	1.59	625	664
5– 6	22nd	50.22	1.68	1.59	653	690
6– 7	23rd	50.00	1.67	1.59	655	688
7– 8	24th	49.70	1.67	1.59*	651	684
8– 9	25th	49.40	1.66	1.58	637	670
9–10	26th	49.10	1.65	1.57	695	731
10–11	27th	48.78	1.64	1.57	673	703
11–12	28th	48.52	1.64	1.56	676	711
12–13	29th	48.19	1.63	1.55	691	726
13–14	30th	47.79	1.62	1.54	698	734
14–15	31st	47.47	1.61	1.53*	701	737

* Body surface for days on which photographs were obtained, i.e., April 13, 16, 23, 30, and May 7 and 14. Other values obtained by direct interpolation.

great. Had the body temperature fallen materially the explanation of the decrease in the heat production could easily be made on the basis of difference in temperature potential. No such explanation is, however, at hand.

The prerequisite conditions for the application of the surface law as outlined by Rubner (34) are somewhat difficult to interpret. If a strict insistence is laid upon like physiological conditions, such as nourishment, climatic influences, temperament, and capacity for work, Rubner would doubtless maintain that the law could not be expected to apply under the conditions of prolonged fasting. Yet these conditions are by no means incomparable with the conditions commonly existing with the majority of pathological subjects with whom enormous variations in the previously mentioned physiological factors are invariably found. A closer examination of the conditions existing during prolonged fasting is not without interest in connection with the treatment by other writers of the results of our studies of the metabolism during diabetes.

In reporting an extended series of observations made in the Nutrition Laboratory on patients with severe diabetes, we compared the metabolism of the diabetics with that found in a series of observations with normal individuals of like height and weight, i.e., of a somewhat thin and emaciated type. As a result of this comparison and the marked difference in metabolism found with diabetics when acidosis was present as compared with that when it was diminished or absent, we asserted that diabetes increased the metabolism approximately 15 to 20 per cent above that of the normal individuals with whom the diabetics were compared. In a discussion of our assertion in which the wholly arbitrary basis of comparison was used of a normal standard value obtained with a large number of individuals of whom the greater proportion were in full vigor, it has been contended (35) that the emaciated diabetics with acidosis showed little or no increase in metabolism. If it is erroneous to apply the surface area law to a normal subject throughout a prolonged fast, it is difficult to see the validity of applying it when there are such marked variations in conditions of nourishment and bodily vigor as existed between the large group of normal persons and the group of emaciated diabetics. In fact, it has been demonstrated that when the diabetics are without acidosis as, for example, when following the remarkable Allen treatment, the metabolism is distinctly lower (36) than with acidosis, so that unquestionably the acidosis *per se* materially increases the metabolism.

With the fasting individual it is evident that, judged by the Rubner specifications, the body surface law may not be expected to obtain; indeed, we find it does not obtain. Nevertheless, it should be pointed out that the differences in the fasting man at the beginning and end of the fast are by no means so great as the differences between pathological individuals, including diabetics, and the average normal vigorous individuals from whom the standard of comparison proposed by other writers has been derived.

With the Meeh formula the results obtained for body surface were clearly questionable, especially when the formula was applied to a body of changing weight; but in view of the accuracy of the Du Bois linear formula, which has been confirmed by the photographic method, we may now accept with full confidence the later measurements of body surface for this individual; hence the values obtained for the heat production as given in the last column of table 1 probably represent with a high degree of accuracy the heat production per square meter of body surface per 24 hours under approximately comparable conditions.

To account for this great difference of 28 per cent in the heat production per unit of surface area, i.e., a physiological measurement that not only is presumed in general to give constant results with all classes of individuals, if not indeed with all warm-blooded animals, but is now specially advocated as the standard for measuring variations in the metabolism in pathological cases, is by no means a simple task. It is certainly clear that the heat production of this individual was in no sense determined by his skin area and the loss of heat from the skin. Variations in body temperature also fail to account for the change in the heat production. The fact that there was in the last week of the fast a rise in the metabolism accompanied by a rise in the pulse rate lends strong credence to the belief that there was present a stimulus to the metabolism which was in a way indicated by the pulse rate; hence we must reiterate (37) our belief that of the numerous factors affecting metabolism, the internal stimulus to metabolic activity is one of the greatest.

SUMMARY

1. An historical consideration of the development of the idea of proportionality between the body surface area and heat production shows that fundamentally it was originally based upon Newton's law of cooling. The idea of a causal relationship between body surface

and heat production is strongly emphasized in foreign writings and distinctly to be inferred from those of a number of American writers. The long existing doubts as to the validity of the older methods for the measurement of body surface have been fully substantiated by the development of the linear formula of the Du Boises, which gives measurements with a high degree of accuracy.

2. Employing a photographic method recently devised for measuring the body surface, the body area of the subject of a 31-day fasting experiment was re-determined and used in recomputing the heat production per square meter of body surface. The values obtained showed a decrease similar to that previously found. The decrease in the heat production per square meter of body surface amounted to 28 per cent, a decrease that can be interpreted only as proof of the inapplicability of the surface area law to subjects with widely varying states of nutrition. This shows it to be impossible to compare a standard value obtained with a large number of robust, normal individuals with that obtained with emaciated diabetics, and thus supplies strong proof of the legitimacy and practicability of the group system of comparing pathological cases with normal individuals of like height and weight, i.e., of general anatomical and morphological similarity.

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THE INTER-AURICULAR TIME INTERVAL

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INTRODUCTORY

In 1910, Wybauw (1) and Lewis (2) working independently, demonstrated that in the mammalian heart, the point of initial excitation corresponds to the location of the sino-auricular node. This finding has been confirmed by Eyster and Meek (3) and others. Although Erlanger (4) and his co-workers have placed a different interpretation on the records of the above named investigators, the view is generally held that the normal seat of impulse formation, is the sino-auricular node and more particularly the head of this node. Granting that this view is essentially correct, the excitatory process is then propagated from this area by muscle tissue to all parts of the heart. If the rate of propagation through auricular muscle is the same throughout, it may be expected that parts at a sufficiently greater distance from the sino-auricular node than nearby parts, would show an appreciable time difference in the onset of their contraction. Thus the left auricle, though in direct and common muscular connection with the right auricle, may be expected to contract later than the right. It is generally taught, however, that the contractions of the two auricles are synchronous.

Yet Chauveau (5), in 1894, stated that "in the horse, the right auricle begins its systole an appreciable time before that of the left auricle." Later Fredericq (6) took up the question and demonstrated the same fact in the dog's heart. Fredericq's pupils—Schmidt-Nielsen (7), Stassen (8)—confirmed his findings. The correctness of this statement has, however, been disputed by Hirschfelder and Eyster (9) who attribute Fredericq's results to the use of excised hearts; they believe that the heart in situ maintained under normal conditions, does not show any time interval in its auricular contractions and that such time

interval occurs only in dying hearts or in hearts suffering in their nutrition. The later work of Fredericq and his pupils, was done on hearts in situ. Hirschfelder and Eyster's failure to demonstrate any time interval between the two auricular contractions may have been due to the use of a too slowly moving kymograph and of too small animals. Leontowitsch (10) likewise disagrees with Fredericq and finds that contractions of the two auricular appendages as well as of the atria may occur in various sequences, although in the majority of his experiments the right atrium and auricle contracted before the homonymous parts on the left side. Recently, Lewis, Meakins and White (11), tracing the course of the excitatory process through the auricles by the method of primary negativity, came to the conclusion that "certain portions of the right auricle contract before certain portions of the left auricle, and vice versa;" that it is therefore "unprofitable" to discuss whether it be the right or the left auricle that first contracts. While individual parts of the auricles doubtless begin their contraction at different times the point of interest insofar as the efficiency of the auricles as feed pumps is concerned, relates to the resultant of these differently timed contractions.[1] In view, therefore, of these contradictory findings it has been thought worth while to submit again the question to experiment by the usual graphic (suspension) method.

METHOD

Dogs of fairly large size—7.7 to 21 kgm.—were employed. They were anaesthetized with ether; no other drug was used. The vagus nerves were exposed in the neck and loose ligatures passed around them. The sternum was removed after ligation of the internal mammary arteries. The phrenic nerves were cut; the pericardium split open and its edges stitched to the sides of the opening in the thorax. Artificial respiration was started meanwhile and maintained throughout the

[1] It was realized early in the course of the experiments that the auricular myogram as obtained by the suspension method could not be depended upon to give an indication of the relative *duration* of right and left auricular systoles, still less any difference that might exist in the onset and duration of the period during which the blood is forced into the ventricles. Since then Wiggers (12) has published an interesting paper bearing on the relation between the auricular myogram and what he calls the "dynamic period" of the systole. While the relative *duration* of right and left auricular systoles may not be obtainable by the suspension method, the relative *time of onset* of the contractions is quite accessible to the method.

experiment. The mixture of ether and air was kept in the neighborhood of 37°C. by means of Brodie's air warmer and anaesthetizer. The heart was prevented from drying by dropping upon it warm Ringer's solution. Cooling of the exposed heart was prevented by means of an electric lamp placed an appropriate distance above it.

The tip of the right and left auricular appendages were connected to receiving tambours by means of François Franck pincettes. These tambours were covered with exceedingly thin and very elastic rubber dam (condom).

Rubber tubes of equal lengths joined the receiving to recording tambours provided with very light levers (thin straw and aluminum writing points). The recording tambours were placed upside down so that the curve of contraction is positive. An electro-magnetic tuning fork giving one hundred vibrations per second and a stimulation signal were mounted beneath the tambours. All writing points were adjusted to the same perpendicular line. The Hürthle kymograph was arranged for a speed of 180 mm. per second with the exception of experiment 1 where the speed was 320 mm. per second.

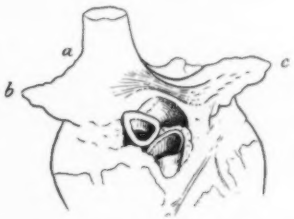

Fig. 1. Sketch of the dog's heart —ventral view—to show the position in which the auricular appendages were placed for the measurements mentioned in the text. The inter-auricular band is shown also.

At the conclusion of the experiment, the heart was removed and the following measurements were taken (*a-b*) distance from the position of the head of the S-A node (auriculo-caval angle) to the tip of the right auricular appendage; (*a-c*) distance from S-A node to the tip of the left auricular appendage (see fig. 1). Great care was taken in making these measurements not to stretch the heart tissue although it was necessary to raise the appendages; even with the utmost care, such measurements are liable to error and must therefore be considered as merely approximate.

RESULTS

The beginning only of each experiment will be discussed in this paper. The study of the effect of vagus stimulation has not been completed and its report must be postponed. By confining the observations on the inter-auricular interval to the beginning of the experi-

ment, the criticism of cooling of the exposed heart and interference with nutrition through prolonged etherization, must be largely overcome. I may say, however, that usually, later phases of the experiment show but insignificant prolongation of the inter-auricular time interval.

A reference to table 1 shows that the average inter-auricular time interval in twelve dogs ranging in weight from 7.7 to 21.36 kilos was 0.013 second (fig. 2). This, therefore, corroborates the findings of Fredericq and his pupils. Moreover, the experiments which form

TABLE I

NUMBER OF EXPERIMENT AND DATE	WEIGHT	RATE OF HEART BEAT	INTER-AURICULAR TIME INTERVAL	NUMBER OF MEASUREMENTS	A. DISTANCE FROM AURICULO-CAVAL ANGLE TO TIP OF RIGHT AURIC. APPENDAGE.	B. DISTANCE FROM AURICULO-CAVAL ANGLE TO TIP OF LEFT AURIC. APPENDAGE.	B-A
	kgms.		*seconds*		*mm.*	*mm.*	*mm.*
1. May 27, 1915.....	19.6	159	0.0130	34	29	54	25
2. June 25, 1915.....	15.7	285	0.0103	67	24	48	24
3. July 3, 1915.....	12.0	136	0.0142	15	28	45	17
4. July 30, 1915.....	10.1	276	0.0105	14	27	42	15
5. August 5, 1915.....	13.7	237	0.0102	12	25	50	25
6. January 4, 1916.....	8.45	175	0.0133	24	22	53	31
7. January 6, 1916.....	10.7	158	0.0132	20	30	60	30
8. January 19, 1916....	11.3	203	0.0100	32	29	55	26
9. January 21, 1916....	7.7	220	0.0100	11	29	44	15
10. January 24, 1916....	8.6	172	0.0137	11	23	40	17
11. February 2, 1916....	12.8	182	0.0169	32	32	52	20
12. February 9, 1916....	21.366	145	0.0165	60	41	76	35
Averages.....			0.013		28.2	51.5	23.3

the basis of this report have been remarkably uniform throughout; no such variations in the sequence of contraction as have been mentioned by Schmidt-Nielsen (loc. cit.) and by Leontowitsch (loc. cit.) were observed at any time.

It may be of interest to note in connection with the figure just given as the average inter-auricular time interval, that practically the same figure can be obtained from one of Lewis' tables (11) dealing with conduction time from S-A node to various points on the auricles. The conduction time from S-A node to the right appendage in 11 dogs was 0.0314 second; the distance was 28 mm. The conduction time from the node to the left appendage in 7 dogs was 0.0446 second; the distance

was 44.6 mm. The difference in conduction time from the S-A node to the right and left auricular appendages respectively is therefore 0.0132 second, a figure which is practically identical with the one given here. It is difficult to state what significance, if any, attaches to this similarity in time differences. The contractions recorded in the manner described in this paper, doubtless give a record of the action of the greater mass of the auricle whereas Lewis' work was confined to a determination of the time of onset of excitation of a very limited part of auricular muscle relatively to the S-A node. If, as we must believe,

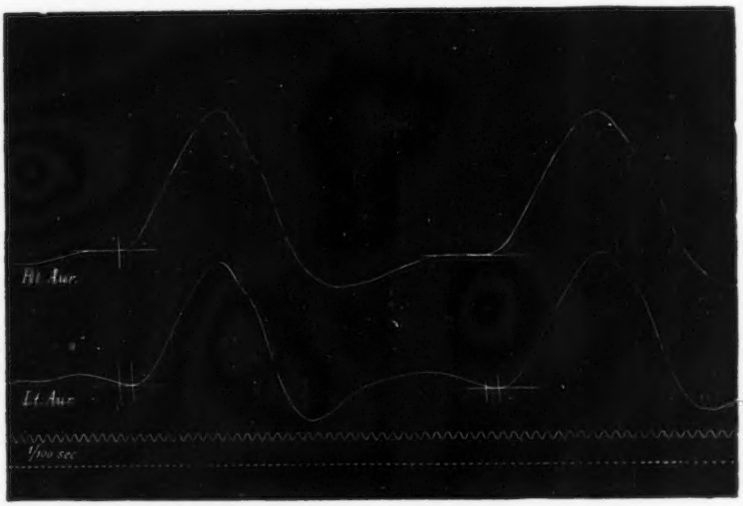

Fig. 2. Tracing of right and left auricular contractions obtained from dog 1.

the contraction of the auricle as a whole is the resultant of the differently timed contractions of its parts, the inter-auricular time interval obtained by recording the auricular contraction should be less than that obtained with the electrocardiograph, the contact points being S-A node and tip of appendages. The results presented in this paper may be so interpreted if reliance may be placed upon the measurements of the heart, for while in Lewis' animals the average difference in distance to be traversed by the excitatory wave was 16.6 mm., the difference in distance in our animals was 23.3 mm.

It occurred to the writer that in view of the solidarity of the auri-

cles, a special structure might exist whose special function would be that of inter-auricular conduction. An examination of the heart shows that a distinct band of muscle tissue stretches from the right auricle to the base of the left auricular appendage and forms apparently the most direct connection between the auricles. The same observation was made by Lewis, Meakins and White (loc. cit.) who call attention to the straight course taken by the fibers of this muscle band. This band has appropriately been called the inter-auricular band.

Fig. 3. Dog's heart hardened in the diastolic state to show the relations of the inter-auricular band. Natural size.

When the heart is hardened with 10 per cent formalin under a pressure of 30 cm. of the solution, the form and relations of the inter-auricular band are well brought out (see fig. 3).

The inter-auricular band. In a dog's heart of average size the right extremity of the band originates about 5 mm. to the mesial side of the auriculo-caval angle and in a direct line with it. This extremity of the band is immediately back of the aorta and spreads in a fan-like manner onto the mesial (right) auricular wall and the root of the su-

perior vena cava. From this origin the band sweeps in an almost straight line to the base of the left auricular appendage but, so far as superficial appearance indicates, its fibers can be traced to near the tip of the left appendage. The band is thinnest near its origin on the right side where it has an angular appearance. It then broadens gradually as it passes to the left auricle. The band is slightly concave from right to left, the concavity being directed upward. It has a length of about 20 mm.

THE EFFECT OF CLAMPING THE INTER-AURICULAR BAND

If this band is of special importance in conduction its compression should lead to at least a lengthening of the inter-auricular time interval. A number of experiments were accordingly conducted in which the band was crushed about its middle between the jaws of a hemostatic forceps, care being taken not to injure the auricular tissue either above or below the band.

Extracts from the Protocols

January 4, 1916. Dog 6. After registering the auricular contractions for five minutes, an attempt was made to clamp the band by sliding a hemostatic forceps against the left wall of the pulmonary artery, the forceps being slightly inclined toward the right of the animal. The first attempt failed, a part of the auricular appendage near the base being compressed instead of the band proper. There was no apparent effect following this attempt. A second attempt succeeded. The inter-auricular time interval lengthened from an average of 0.0133 second to 0.98 second, many measurements showing an interval of 0.1 second (fig. 4). The inter-auricular time interval was therefore lengthened 73.68 times by the crushing experiment.

At the conclusion of the experiment examination of the heart showed, besides the compression at the base of the auricular appendage already mentioned, that the band was crushed transversely, the part crushed measuring 5 mm. The portion of uncompressed auricular tissue below the band measured 4 mm.; the portion above the band measured 10 mm.; these are external measurements and do not, of course, comprise any part of the auricular septum that may have been affected by the clamping.

January 6, 1916. Dog 7. In this and subsequent experiments the contractions of the left ventricle were registered simultaneously

with those of the auricles. The first attempt to clamp the band was successful. The inter-auricular time interval passed from an average of 0.0132 second to 0.053 second an increase, therefore, of 4 times the normal average (fig. 5). The differently timed contractions of the two auricles were quite appreciable to the eye. The average rt.A–lt.V interval at the beginning of the experiment was 0.082 second; there was a gradual increase in this interval toward the close of the experiment, and it was 0.09 second just before clamping the inter-auric-

Fig. 4. Tracing from dog 6 showing the effect on the inter-auricular conduction time of injury to the inter-auricular band.

ular band. This interval after clamping showed an average of 0.102 second.

Examination of the heart showed that the forceps was applied on the band at about the same point as in the preceding experiment, this point being immediately to the left of the location of the auricular septum. The blades, however, included a little more of the tissue above and below the band. Viewed from the interior of the auricles there was no evidence of the compression on the right side of the auricular septum; but on the left side evidence of the compression was seen half-way between the opening of the right pulmonary vein and the

opening leading into the left auricular appendage. The compression affected, in part, the roof of the auricle above the septum and, in part, the septal wall in front of the position of the fossa ovalis.

January 19, 1916. Dog 8. The inter-auricular time interval at the beginning of the experiment was 0.01 second; the heart rate 203 per minute. After section of the right vagus the heart rate rose gradually to 257 per minute; at the same time the inter-auricular time inter-

Fig. 5. Tracing from dog 7; it shows the usual effect of clamping the inter-auricular band.

val gradually decreased to 0.005 second. This was the time interval just before clamping. The clamp was applied in the same position as in dog 7; the inter-auricular time interval immediately after clamping and thereafter, had an average of 0.023 second an increase of 4.6 times the average observed before clamping. There was no appreciable change in the rt.A–lt.V interval, the average being 0.07 seconds.

The band was less well developed than usual; moreover, it had not

been so well crushed by the forceps as the blades had failed to come completely together.

January 21, 1916. Dog 9. An attempt to clamp the band with Erlanger's clamp failed, the clamp being found imbedded in connective tissue on the side of the pulmonary artery. The experiment came to a premature close owing to an extensive tear in the left auricular appendage caused by an accidental pull on the pincette.

January 24, 1916. Dog 10. The band was clamped with Erlanger's clamp, but as it caused hemorrhage it had to be removed and the forceps applied. There was but slight increase in the inter-auricular time interval, viz., from 0.0137 second to 0.019 second—an increase of 1.38 times. The rt.A–lt.V interval had an average of 0.09 second. The records were obtained with difficulty owing to the restlessness of the animal (hemorrhage?).

The band and the auricular tissue immediately adjacent were found crushed by the forceps.

February 2, 1916. Dog 11. Immediately on clamping the band the inter-auricular time interval passed from 0.0169 second to 0.047 second—an increase of 2.8 times. The rt.A–lt.V interval maintained an average of 0.07 second throughout.

The band was found clamped a little to the left of the position described in the previous experiments, namely just above a connective tissue band which binds it to the base of the left ventricle (this connective tissue band is shown in figure 1).

February 9, 1916. Dog 12. Immediately after clamping the band the inter-auricular time interval passed from 0.0165 second to 0.052 second—an increase of 3.15 times. The rt.A–lt.V interval had an average of 0.07 sec. throughout.

The band was found clamped about its middle.

The results have been fairly uniform, in that, crushing of a portion of the band has invariably brought about a distinct interference with conduction as shown by the lengthening of the inter-auricular time nterval from its average of 0.013 of a second to about 0.05 of a second. If we except the experiment on dog 10 in which there was considerable hemorrhage and the reading of the tracings was difficult, and the experiment on dog 6 where not only the band but also the base of the auricular appendage were crushed, it will be seen that the conduction time following injury to the band was from 3 to 4.6 times greater than the normal average.

The fact that the fibers of the band run a straight course from the region of the sino-auricular node to the left auricle and the recent findings of Lewis, Meakins and White (loc. cit.) that the highest rate of propagation of the excitatory process in the auricles is along this band (1252 mm. per second) offers an obvious explanation of the effect of injury to this structure. That it is the most direct and rapid route for the excitatory process is likewise demonstrated by comparing the effects of crushing the entire inter-auricular junction as described by Fredericq (12) with the effects of crushing the band alone as described here. Fredericq found that a complete crushing of the junction causes a complete dissociation of auricular action, the right auricle beating at its customary rate while the left auricle beats at a lower rate, viz., that of the idio-ventricular rhythm. The same phenomenon had been observed by Erlanger and Blackman (13) under different experimental conditions. When the compression is moderate the inter-auricular allorhythmia is temporary, each contraction of the right auricle is followed by a left auricular contraction but the inter-auricular time interval is considerably prolonged. In a tracing illustrating this condition the time interval is about 0.08 second. It would seem, therefore, that of the entire inter-auricular junction, the inter-auricular band constitutes the most important conduction path.

The clamping experiments here reported have demonstrated, I believe, the special importance of the inter-auricular band as a path of conduction between the auricles, for in all the experiments there was a considerable amount of uninjured tissue. This band, however, has not the same importance as a conducting medium as the auriculo-ventricular bundle for while, in the latter, crushing leads to a complete block, in the former, the same injury causes only a prolongation in the time of conduction.

SUMMARY

The time of onset of right and left auricular contraction, contrary to general belief, is not synchronous.

The excitatory wave originating in the sino-auricular node reaches the right auricle sooner than the left; hence the right auricle contracts an appreciable time before the left. The time difference averages 0.013 second.

The most important path of conduction between the two auricles appears to be the inter-auricular band. This special importance is

demonstrated by the effects of crushing the band, the conduction being delayed 3 to 4.6 times the normal average.

The inter-auricular band has not the same importance relatively to the auricles that is possessed by the auriculo-ventricular band, as crushing does not cause a complete block. Its importance lies in the circumstance that its fibers form the most direct inter-auricular path and that the rate of conduction is highest along this path.

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V. DIFFERENCES IN IRRITABILITY AND LATENT PERIOD IN DIFFERENT PARTS OF THE WALL OF THE STOMACH

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I have proposed in a recent paper to study the stomach from the point of view that it has been evolved from a simple tube much as the heart has been enlarged and specialized (1). Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of the primitive intestinal tube along the lesser curvature. These theoretical considerations led to the discovery that strips of muscle from different parts of the stomach wall vary markedly in their tendencies to rhythmic activity, in the rates of contraction, and in the forms of the curves. The most rhythmic region was found on the lesser curvature next to the cardia. The rate of contraction was generally faster and the rhythm more regular in the strips from the lesser curvature than in those from the greater curvature.

Work was done next to see whether differences could be found in irritability and latent period in different parts of the stomach wall, associated with the differences in contractility and based perhaps upon the same developmental peculiarities. Very little could be found in the literature bearing upon the subject. Meltzer (2) observed in dogs and rabbits that faradic stimulation of the outer surface of the stomach in situ gave different results in different regions. The fundus was quite unresponsive. Contractility seemed to improve as he approached the pylorus. Stimulation from the inside of the stomach had no effect except with the strongest currents. A local contraction of the small intestine could be obtained with a much weaker current than that needed for the stomach.

Lüderitz (3) hoped to explain the origin of gastric peristalsis by similar studies. Using mechanical, chemical and electrical stimuli, he noticed that the contraction often appeared cardially to the place stimulated. He thought there was a greater irritability near the lesser

curvature than elsewhere. The reactions were very different in the pyloric antrum. When the stimulus was applied near the pylorus, there was a uniform contraction of the whole distal region. In dogs the fundus was quite insensitive to faradism.

Ducceschi (4) studied the irritability of different parts of the interior of the dog's stomach, using balloons with a device designed to apply the stimulus to the region distended. In the body of the stomach, the effects of mechanical stimulation were weak and slow to appear. He thought the reactions were quicker and more energetic at the pylorus than at the cardia. With the faradic current, the cardia proved to be the most irritable region, and its latent period was shortest. Such stimulation at the cardia sometimes caused inhibition when considerable activity was present. The fundus reacted very similarly but was much less irritable. The response of the pyloric antrum was even less than that of the fundus.

Weak acids had a more pronounced stimulating effect on the cardia than on the fundus. In the pyloric antrum, the effect was generally reversed, the active movements being inhibited. The presence of the balloon in the antrum almost always gave rise to active peristalsis. This was not the case in the fundus. When the antrum was contracting rhythmically, it was quite refractory to outside stimuli. This refractoriness of the active antrum was noticed by Rogers and Hardt (5) while studying the means of inhibiting hunger contractions. Carlson (6) has remarked that the movements of digestion are mainly in the pyloric part; the hunger contractions in the fundus. "Either these two regions of the stomach react differently to local stimulation of the gastric mucosa or else the nervous mechanisms concerned . . . . are different." May (7) found the inhibitory effect of vagus stimulation more pronounced on the cardiac than upon the pyloric end of the stomach.

A very suggestive paper is that of Barbera (8) who found that when the stomach of a frog was stimulated at any point by weak faradic shocks, the contraction appeared first at the cardia. By inserting a small manometer in the cardia, and by resting heart levers on different parts of the stomach, he found that this was due to a shorter latent period at the cardia. With the same strength of current a rise in the tube appeared 8.4 seconds after stimulating the cardia; 10.2 seconds after stimulating the middle region and 13.5 seconds after stimulating near the pylorus. In only one case, while stimulating the pyloric region, that part contracted first and then the cardia, followed by the

middle. Ordinarily, stimulation anywhere on the stomach was picked up first at the cardia, and this insured the downward course of the peristaltic wave.

RESULT OF EXPERIMENTS

This work of Barbera on the frog seemed to me so important and fundamental that I have repeated it. Difficulties with the manometer method caused me to study the stomach in situ in pithed frogs, noting the time elapsed between stimulation and contraction by counting the seconds ticked off by a pendulum. The current was obtained from a Harvard Apparatus Company's inductorium (9) with one dry cell. Platinum electrodes were used, 3 mm. apart. The current was just perceptible to the tongue with the secondary coil at 12.5 cm. Stimulation was kept up for ten seconds, or until a contraction appeared. Care was taken to keep the preparation moist. The work was done in the months of September and October.

Although my results in ten frogs were not so uniform as those of Barbera, the cardia certainly gave by far the quickest reactions. There was generally less difference between the latent periods of the middle and pyloric regions than between those of cardia and middle region, and on four occasions the antrum reacted even more quickly than did the middle. This might have been caused by temporary changes in irritability due to previous stimulation or to the passage of spontaneous waves. The differences in latent period were brought out best by the weakest currents that would take effect in the antrum. Still weaker stimuli showed that the cardia was the most irritable part of the stomach, as it generally responded to currents that had no effect on the middle or on the antrum. Stimulation of the cardia was also more likely to cause a strong contraction of the whole stomach than was stimulation of the antrum. Frequently irritation of the pyloric region brought about contraction at the cardia and in the duodenum before any effect was observed at the point stimulated.

EXPERIMENTAL OBSERVATIONS ON THE STOMACHS OF RABBITS, CATS AND DOGS

A similar technic was followed with the stomachs of rabbits, cats and dogs. The rabbits were killed with a blow on the head; the other animals by etherization and bleeding. The stomach was removed quickly and placed in a shallow dish containing Ringer's solution at

38°C. The upper surface, while being tested, was kept moist by pouring on more warm Ringer's solution. Unfortunately, some cooling could not be avoided. The latent period in almost all of the experiments was determined visually, as has been described. Graphic records were obtained from a few stomachs, enough to show that the simpler technic was sufficiently accurate. These stomachs were suspended vertically and the region to be tested was picked up by two tiny serrefines; the lower one attached to a rigid support, the other to the writing lever. Such methods could not be used regularly on account of the time that would be consumed in making the fifty or more observations required on each stomach. It was feared that during this time there might be such changes in the irritability that no comparison could be made between the observations taken first and those taken last.

The results obtained on any single stomach would have served to illustrate what I wish to show, but it seemed best to average the observations made in the different regions. Figure 1 shows the location of these regions on the anterior surface of the rabbit's stomach, together with the average latent periods for faradic and galvanic stimulation obtained from 10 animals. Figure 2 gives similar data from the stomachs of 8 cats. The faradic current was obtained from the same apparatus that was used on the frog. The secondary coil was kept at 8 cm. in all the experiments. The galvanic current was just strong enough, 1.7 m.a., to duplicate the faradic effects in the cardiac and middle regions. The same platinum electrodes were used.

In the rabbit, the reaction was practically immediate in the esophagus and over the antrum cardiacum. It is well known that striated muscle reaches to the cardia in rabbits and dogs, but the articles consulted did not give the impression that it spreads over the stomach at all. Doctor Whipple kindly looked at some sections from these animals, and found strands extending 2 cm. beyond the cardiac thickening. This complication was not met with in the cat or man, where the lower fourth of the esophagus is free from striated muscle.

As will be seen from figure 1, the latent period was shortest around the cardia and on the lesser curvature as far as the incisura angularis. Beyond that, in the middle of the anterior surface, there is a zone in which the reactions appeared within a second or two. Outside of that again is an area, along the greater curvature, in which reactions were obtained in from one to four seconds in the more irritable stomachs (often from the younger animals). In some of the less irritable stom-

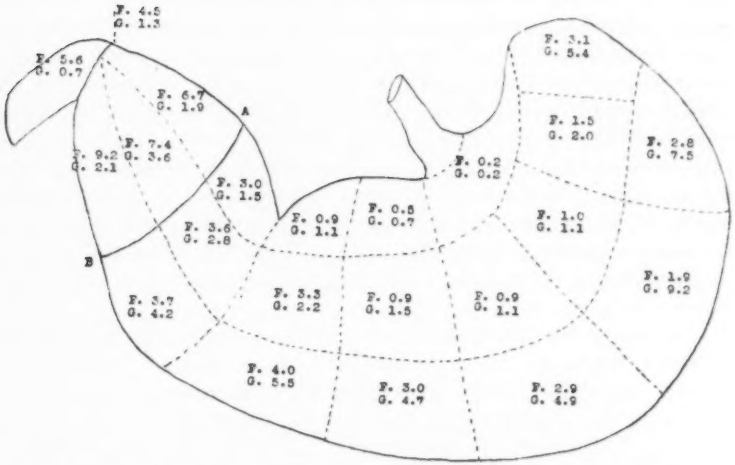

FIG. 1. Anterior surface of the stomach of the rabbit, showing the different regions stimulated, with the average latent periods after faradic and galvanic stimulation. The figures indicate seconds. *AB* is the dividing line between the pyloric antrum and the body of the stomach.

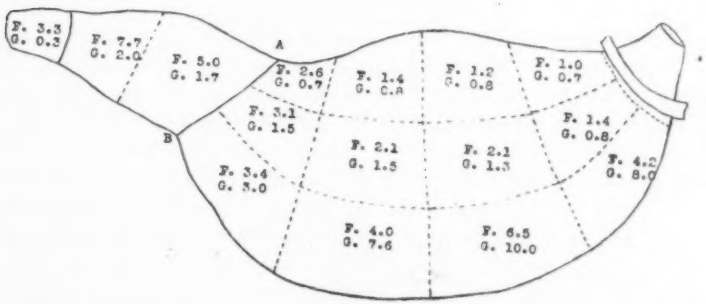

FIG. 2. Anterior surface of the stomach of the cat showing the different regions stimulated with the average latent periods after faradic and galvanic stimulation. The figures indicate seconds. *AB* is the dividing line between the pyloric antrum and the body of the stomach. The other unbroken line represents the pylorus.

achs, no reactions could be obtained at all with the strengths of current used. This comparative sluggishness of the greater curvature was more marked with the galvanic than with the faradic current. Experience showed that if no reaction could be seen after ten seconds, it was useless to stimulate further. The graphic records, however, suggested very strongly that such apparently negative results were more often due to the very small amplitude of the contraction rather than to an entire absence of response. It seemed best, therefore, in making the averages, to represent these results by 10 seconds, assuming that some reaction had occurred by that time. This has given results quite comparable to those found in any single stomach of moderate irritability.

Turning to the charts again, it will be seen that the latent period becomes longer as we approach the antrum pylori. Although the muscle begins to thicken in the preantrum, there is, in the rabbit, a very definite transition from the body of the stomach to the antrum. This can be brought out markedly by leaving the stomach 48 hours in ice-cold Ringer's solution. The dividing line is then very sharp between the gray-blue of the body of the stomach and the red muscle in the pyloric portion. This division is not so apparent in the cat and dog, but it can easily be demonstrated. In all the animals studied, the change in the type of reaction to stimuli was equally sharp at this line. In the preantrum there were deep hour-glass contractions; in the antrum, a centimeter away, there were peculiar local puckerings and tumefactions, often followed by a spasm of the whole pyloric end of the stomach. The latent period for the faradic current was often more than double that observed on the other side of the dividing line. Strange to say, there was no such increase with the galvanic current. This rather striking observation will be discussed later. The lesser curvature reacted a little more promptly than did the rest of the antrum.

It should be emphasized that the pyloric ring showed a shorter latent period than did the rest of the antrum. The duodenum, a few millimeters away, reacted to the galvanic current very promptly—within 0.5 to 1.0 second. For some unknown reason, the duodenum seemed to lose its irritability to the faradic current very rapidly after excision. With the organs in situ, both currents caused the duodenum to contract promptly.

POSTERIOR SURFACE OF THE STOMACH

It was soon noticed that in most stomachs the latent periods were a little longer on the posterior than on the anterior surface, and this conclusion was supported by the averages. This sluggishness was most marked along the greater curvature. The sequence of the four series of tests—with faradic and galvanic currents on both surfaces—was changed daily, although it was soon evident that the irritability of the stomachs remained quite constant for a period longer than the hour required to complete the work. To make sure that differences in thickness or resistance in the peritoneal coat could have nothing to do in altering the reactions, tests were made here and there before and after laying bare the muscle. No differences in latent period could be produced in this way.

EFFECTS OF MECHANICAL STIMULI

Experiments in which the excised stomach was pinched by fine mouse-tooth forceps gave results very similar to those with a weak faradic current. In the rabbit, the region about the greater curvature from the fundus to a point opposite the incisura angularis failed to react. The antrum showed the typical puckering contractions after a latent period of from 3 to 5 seconds. The pylorus and duodenum reacted almost immediately. The region around the cardia was so sensitive that a contraction generally appeared each time the Ringer's solution was poured on the stomach.

DIFFERENCES IN IRRITABILITY

With weaker currents, a close relation could be shown between the degree of irritability and the latent period. With one-fourth the strength of the galvanic current previously used, reactions were obtained (in the rabbit) only around the cardia; with one-half the strength, they appeared within a radius of 3 cm. from the junction of the esophagus and lesser curvature. This also was the only part of the stomach that reacted to the faradic current with the secondary coil at 13 cm. Similar results were obtained in the cat and dog.

Just as in the frog, stimulation, particularly of the middle region of the stomach, often showed itself first as a contraction at the cardia or along the lessser curvature. This might or might not be followed by a contraction at the point stimulated, depending on the strength and duration of the stimulus. This was very marked in the cat.

STUDIES WITH THE STOMACH IN SITU

To make sure that these differences were not due to the trauma of excision or to unequal post-mortem changes in the gastric plexuses, the work was repeated on the stomachs of rabbits, cats and dogs whose abdomens were opened after etherization. The vagi and splanchnics were left intact. The intestines were covered with gauze wrung out in warm Ringer's solution, and the stomach wall was moistened frequently with the same fluid. Regional differences were found corresponding to those in the excised stomachs. The cardia, lesser curvature and pyloric antrum reacted in about the usual time; but, strange to say, the rest of the stomach—the fundus and body—was generally much less irritable than in the excised organ, and often this region would not react at all to the currents used. The possible significance of these findings will be discussed later.

EXPERIMENTS ON THE HUMAN STOMACH

A few experiments performed on the human stomach, while necessarily inconclusive, suggest that similar differences will be found there also. Through the kindness of Doctors Baxter and Brill, I had the opportunity of making the desired tests twenty minutes after death in a man who died of nephritis. Unfortunately, there was ascites with edema of the peritoneum which may have had some influence on the reactions. Both in situ and when excised, this stomach was less irritable than those of animals, and to get reactions with the faradic current, the secondary coil had to be at 6 cm. The cardia and adjacent lesser curvature could not be studied satisfactorily in situ as the pulling that was necessary seemed to affect the reactions. Elsewhere the reactions were very much like those in the cat's stomach. After excision, the stomach contracted down to the cylindrical J form, so well known to radiologists. It seemed to lose its irritability rapidly; the preantral region was the only place which reacted twice in the same way, and no conclusions could be drawn.

Another specimen obtained at operation on a man with beginning malignant degeneration of a small scar at the pylorus showed definitely the difference between faradic and galvanic effects in the antrum; also the much greater irritability of the duodenum as compared with the stomach. Through the kindness of Dr. W. I. Terry, I was able to study the reactions in the pyloric half of another human stomach during an operation for an old cholecystitis. The anesthetic was gas-oxygen

and the abdominal wound was infiltrated with novocain. The only definite statements that could be made after this work were that the stomach was less irritable than that of the animals; that it was a little more irritable near the lesser than along the greater curvature, and that the irritability of the duodenum was much greater than that of the stomach. Even with the coil at 4 cm., the faradic current failed to bring about a contraction in the stomach.

DISCUSSION

The lesser curvature, and particularly that part near the cardia, is more irritable than the fundus and body of the stomach; and it seems very probable that these differences can be ascribed to the developmental peculiarities outlined at the beginning of this paper. Other differences have been found, however, which show that the distance, spacially or embryologically, from the primitive tube is not the only factor modifying the irritability. As was suggested in the previous paper (10), the muscle of the stomach wall has probably been modified to serve different purposes in different regions. This would explain the big differences which I believe exist between the muscle of the body of the stomach and that of the pyloric antrum. Such differences seem inevitable when we remember the differences in function. While the principal activity of the fundus is to maintain a slight even pressure on its contents, the antrum must contract rhythmically and powerfully for hours at a time. Recently Lee, Guenther. and Meleney (11) have shown how remarkably the diaphragm differs from other muscles in its physiologic reactions and its chemical constitution. These differences are all related to its unceasing activity. For the same reason, we may expect some day to find that the reddish muscle in the pyloric antrum has more sarcoplasm, glycogen, etc., than the pale muscle in the fundus.

The better reaction of the pyloric antrum to the galvanic current suggests that the muscle is more sluggish in this region. One is reminded of the reaction of degeneration in striated muscle, or of such experiments as those of Fick (12) who noticed that an induced current which stimulated a frog's muscle had no effect on a mussel's muscle. The latter responded, however, to a slowly increasing galvanic current which had no effect on the frog. Further experience with excised strips has given me the impression that this sluggishness is due partly to nervous inhibition. If the irritability of the muscle is dependent upon

its nervous connections, we should expect the reactions to become progressively slower after removal of the stomach from the animal, and particularly after the trauma of cutting strips. The opposite is true; not only may the latent period become shorter, but it has been found in a number of instances that strips from the antrum would contract more promptly after they had been in the ice-box 24 and even 48 hours than they did immediately after excision. It has already been noted that the excised strips will generally beat rhythmically on the second or third day better than on the first. Although the enteric nerves are probably the last to die in the body (13), it is hardly conceivable that their functional capacity could *improve* after 48 hours, and we must look for the differences in the muscle itself.

The nervous inhibition with the stomach in situ probably explains Meltzer's failure to get good reactions from the cardiac half of the stomach. As will be shown in a subsequent paper, strips of muscle cut from such a refractory fundus and stimulated in a moist chamber will react markedly to the same current after a latent period of less than one second. Ducceschi has remarked upon the refractoriness of the pyloric region when it is active. It would appear that the nervous mechanism serves more to restrain the muscle than to raise its irritability. Such inhibition may be very necessary to save the stomach from the fatigue and perhaps incoordination that might follow its reaction to every stimulus received during digestion.

It is very probable that there are big differences again in the muscle of the duodenum, which not only contracts so differently, but has a shorter latent period and a greater irritability than that of the antral muscle. This was noticed by Meltzer. Schiff also remarked, years ago, that when the digestive tract is stimulated actively by the cutting off of its blood supply, the stomach, with the exception of the cardia, is the part least affected (14).

The tendency of the antrum to respond as a whole when stimulated at any one spot may have some clinical interest. Roentgenologists occasionally see such spasm in man when an ulcer is located in the pyloric region. As the pylorus is more irritable than the rest of the antrum, it is not surprising that it should often be closed so tightly when this part of the stomach is irritated. Interesting also to the clinician is the tendency to deep hour-glass contraction after stimulation in the preantral region in animals. That is the common location for such spasm in man. This finding is in agreement also with the observation that strips from this region show a far wider amplitude of

rhythmic contraction than do those from any other part of the stomach wall.

I agree entirely with Meltzer that it is quite hopeless to attempt to influence the stomach therapeutically by intragastric or abdominal electricity. The refractoriness of the stomach in situ, particularly when stimulated through the mucous membrane, is so great that even powerful currents will produce no motor effect.

SUMMARY

It has been shown in the frog's stomach that the cardia is the most irritable region; also that the latent period is shortest at the cardia and longest near the pylorus. These peculiarities should have much to do with insuring the aboral course of the peristaltic waves.

The mammalian stomach has been studied from the point of view that it has developed from a primitive tube much as the heart has been enlarged and specialized. Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of this tube along the lesser curvature. These developmental differences probably have something to do with the regional differences in irritability and latent period found in the stomachs of rabbits, cats and dogs.

The latent period for faradic, galvanic and mechanical stimuli is shortest around the cardia and along the lesser curvature as far as the incisura angularis. These limits include also the most irritable part of the stomach. The region of the greater curvature and fundus is much less irritable and often fails to react at all.

There is, particularly in the rabbit, a definite transition from the pale muscle of the body of the stomach to the reddish muscle of the antrum. The change in the type of reaction to stimuli is equally sharp at this line. In the preantrum, there are deep hour glass contractions; in the antrum, a centimeter away, there are small puckerings often followed by spasm of the whole pyloric end. The latent period in the antrum is markedly lengthened for the faradic current; very slightly for the galvanic.

The pyloric ring is more irritable and reacts more promptly than does the rest of the antrum.

The duodenum is much more irritable than the pyloric antrum.

The posterior surface of the stomach is a little less irritable than the anterior surface, and the latent periods are longer.

With the exception of the cardia and lesser curvature, the stomach

in situ is often quite refractory to stimulation in its cardiac half. This seems to be due to nervous inhibition as it is less marked in the excised stomach, and still less so in strips of muscle cut from the fundus or greater curvature.

It seems likely that the nervous mechanism serves more to restrain the muscle—to keep it from reacting to every stimulus—than to render it more irritable.

The little work done so far on the stomach of man has given results in agreement with those obtained in animals.

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THE VEGETARIAN DIET IN THE LIGHT OF OUR PRESENT KNOWLEDGE OF NUTRITION.[1]

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The prevalence of pellagra in the United States during recent years, and the accumulation of evidence that it is induced by a faulty diet, chiefly of plant origin, has aroused an interest never before equalled in the question of the adequacy of the strictly vegetarian diet. In the present paper we desire to discuss the vegetarian diet in the light of an extensive experience in feeding restricted diets to several species of animals.

Testimony as to the favorable results of human experience with what are purported to be strictly or practically vegetarian diets are so untrustworthy as to merit little confidence, because of the frequent consumption of foods prepared with milk, eggs, and butter, and the use of soups, gravies, etc., which include extracts of foods of animal origin. Practical experience furnishes abundant evidence that cattle, sheep, and other grazing animals grow from a comparatively early age entirely upon food derived from plants. We have previously reported considerable success in the nutrition of swine during growth on strictly vegetable mixtures when these contain the leafy portion of the plant (6, 12). We have not, however, found convincing evidence that any mammal has been adequately nourished from weaning time by a mixture of the seeds of plants and has made the maximum amount of growth and at the maximum possible rate. Pigs or rats may grow for a time on such rations, but always, so far as we are aware, at a rate slower than the normal, and they suffer suspension of growth before the normal adult size is reached. A colony of pigeons kept by the Department of Experimental Breeding at the University of Wisconsin has, however, lived several years in an enclosure and has been confined to a mixture of cracked corn, wheat, Canada peas, hemp seed, millet

[1] Published with the permission of the Director of the Wisconsin Experiment Station.

seed, and occasionally kaffir corn. They have been normal in every respect and their breeding powers have not suffered impairment. While pigeons in the open may vary their diet with insects, worms, etc., it seems hardly probable that this could possibly have happened to an appreciable extent in these caged birds. It would appear that the pigeon is not strictly comparable with pigs and rats as to its nutritive requirements.

The earliest well planned effort with which we are familiar to test the adequacy for growth and maintenance of mammals, of a vegetarian diet of wide variety, was made by Slonaker (1). He fed a group of rats on a strictly vegetarian mixture which included not less than twenty representatives of naturally occurring foodstuffs and afforded as great a variety as the human vegetarian in California would be likely to have on his table. Another group which served as controls received the same diet and in addition a small amount of meat two or three times a week. The diets were adhered to from the age of one month to the end of life and careful records were kept of the rate of growth and the degree of activity of all the animals. The vegetarian rats were frail and weak and showed extreme lassitude and indifference. The omnivorous controls were the reverse in all respects. They had an average span of life of 1020 days as compared with 555 days for the vegetarians. Slonaker concluded from a review of the literature relating to the subject on man and animals that similar results would be obtained if man were subjected to a strictly vegetarian regime throughout his lifetime.

Our own experiments in which rats were fed a wide variety of vegetable foods confirm in all respects the results of Slonaker. We did not attempt to keep our rats beyond the age of six months since our interest lay in their ability to make such a choice of foods as would best meet their nutritive requirements. The rats whose records of growth are shown in Chart 1, Lot 416 received corn, wheat, oats, rye, cooked beans, green peas, wheat embryo, corn gluten, and wheat gluten, flaxseed oil meal, green clover and green alfalfa, onions and peanuts.

Like Slonaker's rats they grew at about half the normal rate, but ceased to grow after the first ninety days. They appeared to be in a fairly good condition for a time thereafter, but were of course pigmies beside normal rats of the same age. One female even produced young, and did the best she could to suckle them, but at ninety days of age they weighed but 35, 39, 39, 61, and 36 grams respectively.

It is safe to say in the light of the results which were secured in this experiment as contrasted with those more recently obtained with cer-

tain simple vegetable mixtures in which no choice was permitted, (Charts 2, 3, 4,) that the instinct of the rat is not a safe guide to the selection of foodstuffs.

Voegtlin in 1914 (2) reported that rats, mice, and monkeys were unable to maintain their health for more than a brief period on a diet of corn, carrots, sweet potatoes, and oats. He observed, frequently in three or four days, lesions such as hyperemia and hemorrhage in the gastro intestinal canal. Sometimes the kidneys, lungs, and other organs showed congestion and slight hemorrhagic condition.

Later studies by Koch and Voegtlin (3) have shown remarkable changes in the chemical composition of the nervous system of monkeys and rats fed exclusively on corn embryo which had been deprived of most of its fat by pressing. They state that the corn embryo is poor in substances preventing beri-beri. They have observed similar results when monkeys were fed corn meal alone, corn meal and sweet potatoes, or raw carrots as the sole source of nutriment.

Goldberger (4) has contributed further evidence tending to incriminate the vegetarian diet. Voegtlin, following the suggestion of Funk, expressed the belief that the production of pellagra is the effect of the deficiency of certain unidentified substances to which Funk gave the name vitamines, but he also suggests the presence of toxic substances such as aluminum in vegetable foods and a deficiency in the diet of certain amino acids as possible causes contributing to the production of the symptom complex seen in pellagra. Funk (5) believes in the existence of a number of vitamines, one preventing scurvy, another beri-beri, another pellagra, and has suggested the probability of the various species, e.g., birds and mammals requiring different vitamines.

Experiments conducted at the Wisconsin Experiment Station since 1906 and published in part in 1911 (6) revealed for the first time the evil effects for cattle of diets restricted to the wheat plant and the oat plant respectively, while the entire corn plant forms a perfectly adequate ration. The Experiment Station literature furnishes numerous reports of the failure of the corn kernel alone to properly nourish pigs during the growing period. Hart, Miller and McCollum have recently discussed the production of a condition in pigs which is histologically closely similar to if not identical with polyneuritis on diets containing a considerable variety of vegetable foods supplemented with minimal amounts of foodstuffs of animal origin (6). Holst (7) and his co-workers have reported the failure of guinea pigs to maintain life on oats as the sole food, whereas oats and cabbage proved adequate for the mainte-

nance of well-being. This he attributed to a lack of an antiscorbutic substance which is supplied by the cabbage.

Young rats of 30 to 50 grams weight are unable to grow or to long maintain life when fed exclusively on one from the following list of vegetable products: Corn, wheat, oats, barley or rye kernel, polished rice, peas, beans, cottonseed meal, cottonseed flour, alfalfa, corn germ, wheat germ, flaxseed oil meal, peanuts, onions, cabbage, corn gluten, or wheat gluten. It is of great interest, therefore, to observe the effect of feeding a mixture of two of these ingredients, either of which alone fails to support growth or maintain well-being in the rat. Chart 2, Lot 478, shows how nearly nutrition approaches the normal expectation when rats are fed a simple mixture of alfalfa flour 40 and polished rice 60 per cent. Two of these rats, the male and one female, reached 83 and 80 per cent of the weight normal for the adult and are still in good condition after ten months on this diet. Two litters of young were produced by one of the females, but were not reared.

Chart 3, Lot 273, illustrates the practically complete nutrition of rats fed on a monotonous diet of ground corn (maize) 50, alfalfa flour 30 and peas 20 per cent. One female (6) has produced three litters of young during the first ten months on this diet and has successfully reared two litters. Her daughter, which never had any other food except that on which the mother had lived, grew to normal maturity (Chart 3, No. 7) and has brought forth a litter of young and successfully reared them, the young appearing perfectly nourished at the present age of twelve weeks. They have been confined to the same mixture which nourished their mother and grandmother.

Chart 4, Lot 271, illustrates the behavior of a group of rats fed exclusively from an early age (about one month) on a monotonous diet of ground corn 50, alfalfa flour 30, unextracted wheat embryo 20 per cent. Their growth was somewhat slower than the normal expectation, but three females have reached non-pregnant weights of two hundred grams, which is somewhat greater than that of many animals in our breeding stock which receive a mixture of corn 30, wheat 30, oats 30 and flaxseed oil meal 10 per cent, and a liberal supply of fresh milk daily, and clover or alfalfa daily when it can be obtained fresh.

The breeding record of this lot is poor, four females now nearing the end of their period of sexual activity, have produced but three litters, two of these litters being reared. The nutrition of these rats as shown by their appearance is faulty. At the age of one year they appear old and all are suffering somewhat with skin eruptions which cause

scratching. Their hair is coarse and thin. Their ears and tails have remained free from inflammation and hemorrhage. These conditions we have frequently observed in rats fed upon certain rations of inadequate character. Cutaneous horns, often more than a centimeter long, frequently grow upon the noses of rats suffering mal-nutrition. These usually disappear within a month when the animals are placed upon a highly satisfactory diet. Certain dietary deficiencies lead to an inflamed and oedematous condition of the eyes (8).

An adequate working hypothesis concerning the essential factors which operate in the making of a successful food mixture is of fundamental importance as a guide to the study of the nature and extent of the deficiencies of the individual natural foodstuffs. Such a one is, we believe, now available. McCollum and Davis (9) working with diets consisting of purified protein, carbohydrate, fats, and salt mixtures have established the fact that in addition to these dietary constituents there must also be supplied two factors whose chemical natures are at present unknown.

Since, apparently, nothing is known regarding the chemical natures of these dietary essentials, and since the unknown factor which is present in certain fats seems to contain neither nitrogen nor phosphorus, we have for reasons set forth elsewhere (10) proposed to discontinue the term vitamine. This term is now frequently used to include a number of hypothetical substances each of which is supposed to so regulate the metabolism as to prevent a type of pathological functioning. Instead of using this general term to include all such substances, we have suggested the terms fat soluble A and water soluble B (10). The fat soluble A which is essential for growth and certainly in mammals for maintenance as well, was first observed in butter fat, but was later found to be present in many natural foodstuffs. Butter fat and egg yolk fat apparently contain more of it than any other natural foods. Animal tissues contain some of this unknown factor, especially the active organs. It is present in small, but wholly inadequate amounts in the cereal grains. A much greater content of it is found in the leaves of the forage plants, among which we have studied the flour prepared by grinding alfalfa leaves. The vegetable oils generally contain very little, if any, of this substance. It is a curious fact that certain seeds, such as the corn kernel which undoubtedly contains it, since rations so made up that the only factor lacking is supplied by butter fat, are also rendered capable of promoting growth to some extent in the absence of butter fat, by the inclusion of ground corn. Apparently

in the plant cell this substance is not associated with the fats and is not readily removed by fat solvents. Possibly it is in chemical union and is in a form not soluble in fats.

The second dietary factor of unknown chemical composition, the water soluble B, appears never to be associated with the fat fraction as it is isolated from natural foods. It is readily soluble in water and in alcohol, but very slightly soluble in acetone, benzene, ethyl acetate and is insoluble in ether (10).

In our experimental diets we have employed either water extracts or alcoholic extracts of wheat embryo as a source of the water soluble B, since this material is so rich in it that two per cent of embryo in the diet is adequate for growth at the normal rate, provided all other factors are properly adjusted (9). For the fat soluble A, three to five per cent of butter fat suffices. Diets consisting of purified protein, dextrin and salts, which will not induce growth in young rats, likewise produce no growth when one of the additions, wheat embryo extract or butter fat is added, but becomes adequate when both are present (9).

Extracts which contain the dietary factor, the water soluble B, also relieve polyneuritis in pigeons which is induced by diets of polished rice or purified foodstuffs (10). There are many reasons for believing that it is one and the same substance which is concerned in the cure of this disease and in inducing normal maintenance and growth.

We question, for reasons which will appear later, the justification of Funk's assumption of the existence of several unknown chemical dietary constituents. We are convinced that all the observed pathological phenomena can be accounted for in maladjustments relating to the following factors:—the inorganic constituents; poor quality and inadequate quantity of the protein content; shortage of the substances A or B, or of both of them, and in certain cases the presence of toxic constituents in the natural foodstuffs. The latter may be of either inorganic or organic nature. Aluminum and colloidal silica in the one class have been suggested (2) and gossypol of the cottonseed, and the toxic effect of the oil of the wheat kernel which we have recently described (11) may be cited as examples of the second.

We have now tested fully on three natural foodstuffs viz. wheat, wheat embryo, and rice, our working hypothesis which postulates the need of but two chemically unidentified factors A and B as described above in addition to the well recognized constituents, protein, carbohydrate and inorganic salts. These studies make possible the interpretation of the ways in which the vegetable foodstuffs may give rise to metabolic disturbances.

The wheat kernel alone does not induce growth in the rat or the pig (12). By a systematic procedure we have fed to different groups of young rats the following rations: (1) Wheat and suitable salt additions which made the mineral content similar to that of milk; (2) wheat and a purified protein, casein; (3) wheat and butter fat to furnish the fat soluble A; (4) wheat, salts and protein; (5) wheat, salts and butter fat; (6) wheat, butter fat and protein; (7) wheat, salts, butter fat and protein.

It has been definitely established that while rations 1 to 3 inclusive improve the condition of the animals in some degree, appreciable growth or prolonged wellbeing is not possible. Chart 5, Lot 223, illustrates how ration (4) supports growth at the normal rate to nearly normal adult size followed by early failure. Chart 6, Lot 319, illustrates how ration (5) which includes the addition of both salts and butter fat to wheat fails to induce normal nutrition. These rats were very inactive, and this appeared to be the result of depressed sensibility. One was finally killed when in a very feeble state and one lung was found badly infected. The other slowly lost weight after reaching a weight of only 215 grams. Chart 7, Lot 380, shows relatively little growth on ration (6) when butter fat and protein were added to wheat. When failure was imminent and the animals exhibited partial baldness and eczematous condition of the skin, tail, and ears, a salt mixture of suitable composition was added, with the result that two of them promptly responded with rapid growth to the normal adult size and all made complete recovery from their pathological condition. Ration (7) (Chart 8, Lot 223 B) on the other hand is complete and induces growth to the usual adult size at the normal rate and supports reproduction at normal intervals and successful suckling of the young was the rule. These animals had sleek coats and appeared to be perfectly nourished. We have here a complete picture of the dietary deficiencies of the wheat kernel. In addition to proteins of poor quality, an inadequate content of the unknown substance, the fat soluble A, an unsatisfactory inorganic content, wheat also contains a toxic constituent which may contribute in certain rations to the failure of nutrition of the animals. This is brought out by the charts illustrating the cause of nutritive failure of rats fed wheat embryo.

Wheat germ, when fresh, is a fairly palatable food containing about 10 per cent of oil and 30 per cent of protein. In experiments with pigs confined in metabolism cages during forty days and fed a ration, the protein of which was derived entirely from this source, it has been found that as much as 39 per cent of the nitrogen ingested may be retained for

growth. Similiar experiments with the nitrogen of the entire wheat kernel and with skim milk showed retention of 20 and 63 per cent respectively of the ingested nitrogen. The proteins of the wheat germ are, therefore, of good quality. This is borne out by the curves of Chart 9, Lot 397, in which the maximum growth took place on a ration containing but 10 per cent of total protein all derived from this source.

We have described elsewhere the complete series of experiments illustrating the nature of the dietary deficiencies of the wheat germ (11). Three of these are of especial interest in connection with the present discussion. On a ration of wheat embryo alone young rats do not grow at all. A ration consisting of fat free wheat embryo 33, dextrin 51, a salt mixture 11, and butter fat 5 per cent induced growth at a rate exceeding the normal and maintained the animals in a good state for a long period (Chart 9, Lot 397). When the butter fat was omitted from the food mixture the rate of growth was greatly depressed and the animals all failed after two to three months, (Chart 10, Lot 521). When only the salt additions were omitted from the ration of Lot 397 (Chart 9) the rats suffered complete suspension of growth (Chart 11, Lot 585). After seventy days on this diet, all animals responded promptly with vigorous growth when the salt mixture employed with Lot 397 was introduced. A ration closely similar to that of Chart 9, Lot 397, except that unextracted wheat germ was employed (Chart 12, Lot 495) shows the injurious effect of wheat oil. The rats fed this mixture grew at about half the normal rate during the first four or five months, and then rapidly lost weight and died. The improved wellbeing of rats fed the same mixture, but with the fat of the wheat embryo extracted, was very marked. The substances extracted by ether exert a depressing effect on the growth and health of the animals. A ration of wheat embryo leads to failure therefore, although its proteins are satisfactory, because of maladjustments of the relationship of its inorganic constituents; a content of the fat soluble A which is below the optimum, and because of the presence of a toxic substance, part at least of which is extracted with the oil by means of ether.

In a former paper we have discussed in detail the nature of the dietany deficiencies of rice (13). This inquiry was conducted by systematically adding one or more purified food constituents as described above in the case of wheat. Polished rice (heated) does not support growth even when supplemented with purified protein, a salt mixture, and butter fat (Chart 13, Lot 324). There must likewise be added a small

amount of the second unknown dietary constituent which we have described above as the water soluble B (Chart 14, Lot 401). The mixture of rice and purified additions just enumerated becomes a complete ration when there is added to it a small amount of a water or alcoholic extract of wheat embryo or of egg yolk. We now know that active extracts can be prepared from a long list of natural foods including unpolished rice, the cereal grains, vegetables and leaves of forage plants. Polished rice is comparable to a mixture of purified proteins, carbohydrates, and salt. Its protein content is probably too low to support maximum growth and it is poor in inorganic elements and these are probably not present in relations approximating the optimum. In addition, however, it is deficient in both the dietary factors A and B. In contrast to wheat which contains the B in abundance (See Chart 19, Lot 475), but requires three kinds of purified additions, rice requires four kinds to make it complete.

It has been pointed out that the ration of Chart 11, Lot 585, leads to failure because of its unsatisfactory inorganic content. When this is suitably modified by salt additions growth proceeds at the maximum rate (Chart 9, Lot 397). This serves as an illustration of how we can bring animals into a pathological condition and place the responsibility upon a single dietary factor. Chart 12, Lot 495, serves as an example in which nutritive failure follows as the result of the toxic constituent carried by the wheat germ, all other factors being known to be so adjusted as to induce maximum growth and apparently perfect nutrition for a long period. It is now possible to complete the series and describe rations which will lead to nutritive disaster, and to point definitely to each of the other dietary factors discussed above, as being singly responsible for the failure of the animals.

In Chart 13, Lot 324, the ration of polished rice, casein, dextrin, butter fat, a salt mixture and agar-agar, is entirely adequate for growth except that it lacks the water soluble B. Chart 14, Lot 401, illustrates the vigorous growth which ensued when this factor was supplied in the form of a cold water extract of wheat embryo. (Compare Charts 13 and 14). Chart 15, Lot 417, illustrates the failure of rats to grow on a ration of casein, dextrin, a salt mixture and agar-agar, the food mixture carrying an abundance of the water soluble B in its content of water extract of wheat embryo. The cause of this is the lack of the fat soluble A as is shown by Chart 16, Lot 418, which grew at the maximum rate when fed a ration closely similar in all respects, but containing 5 per cent of the butter fat.

Chart 6, Lot 319, shows failure of nutrition due to the low content of proteins of relatively poor quality. All the other factors in the ration are satisfactory and this ration becomes wholly adequate to support normal growth and reproduction when it is supplemented by a pure protein and no other addition (Chart 8, Lot 223B). Without protein addition the span of life was limited to nine to eleven months and the full adult size was never reached.

We are at present engaged in a histological and pathological study of animals whose failure of nutrition was brought about by the methods of feeding described above in which but a single dietary factor operated in inducing a pathological state. It is hoped that these results may materially advance our understanding of the effects of specific starvation.

Among the symptom complexes recognized by clinicians as attributable to inadequacy of diet are beri-beri, scurvy, and pellagra. The first two are now almost universally conceded to be due to a deficiency of a chemical factor in the diet. Hess (14) has reached the conclusion that the symptomatology of beri-beri and scurvy have many features in common.

Concerning pellagra disagreement still exists as to whether it is a dietary disease. The noteworthy achievements of Funk whose extensive investigations of avian polyneuritis entitle his views to respect believes that each of these diseases, beri-beri, scurvy, and pellagra is the specific result of a deficiency in the diet of a specific substance which he termed *vitamine* (15). Funk (5) apparently believes that the existence of three recognizable pathological states all attributable to faulty diet warrants the assumption of the existence of three unidentified chemical factors which form the specific vitamines, each of which when present so regulates the metabolism as to prevent a particular type of abnormal functioning of the cells.

The inadequacy of the sweeping explanation offered by Funk (16) and Voegtlin (17) that pellagra is caused by a lack of vitamines is apparent from Voegtlin's observations on animals fed corn meal and corn meal and sweet potato (3). Failure of nutrition with severe degenerative changes in the nervous system and early death were observed. We present in this connection the curves of growth of a group of rats whose ration consisted of ground corn 71.3, casein 18.0, agar 2.0, salts 3.7, and butter fat 5.0 per cent (Chart 17, Lot 568). The casein was purified to a practically ash free condition and must be regarded as free from *vitamines* as well. Aside from the essential dietary factor

furnished by butter fat all chemical substances of unknown nature which could possibly be termed vitamines are supplied by the corn. These animals are in a state of excellent nutrition at the present time after six months on this diet. We do not wish at this time to discuss at length the nature of the dietary deficiencies of the corn kernel, but we can add that if in the above ration either the casein or the butter fat is omitted the nutrition of the animals is distinctly interfered with. Corn, according to our interpretation, contains a liberal supply of the water soluble B, but less than the optimum amount of the fat soluble A, together with proteins of poor quality, as one of us has already pointed out (18). Its inorganic content is entirely inadequate to support growth (Chart 18, Lot 618). The explanation for the failure of nutrition of the monkeys, of Koch and Voegtlin (3) is, we venture to suggest, to be found in a maladjustment of the well recognized chemical constituents of the diet rather than in "lack of vitamines."

Voegtlin (17) has recently discussed the effect of milling of corn on the removal of the vitamine-containing portion of the grain and has advanced the theory that the modern methods of milling of corn are an important factor in the causation of pellagra in the south. This is probably an important contributing factor, but there is certainly a great tendency to over estimate the importance of the as yet unidentified chemical factors lumped together as vitamines, and to attribute to them an importance paramount to the other factors of the diet. Although his experimental diets have all been entirely too complex to interpret, Goldberger (19) shows a creditable grasp of the situation when he states: "The possibility, if not the probability, of a 'twilight' zone within which a very slight change in any of the dietary components may cause an important shift of balance is not to be overlooked." We have in the present and in former papers described methods of experimentation which enable us to isolate the individual dietary factors and to evaluate in any natural foodstuff the individual dietary components, and have shown how the degree in which the shortcomings of one natural foodstuff are corrected by the supplementary character of another, can be studied by biological methods sufficiently sensitive to yield results of great practical value.

Our practically complete success in the nutrition of rats with strictly vegetarian diets made up of but three natural foodstuffs, and the failure attending the employment of a wider variety in the food mixture, emphasizes the fallacy of the assumption that the safest plan to insure perfect nutrition, is to include a wide variety in the selection of the

constituents of the diet. So long as definite knowledge is wanting concerning the specific nutritive properties of the constituents of the diet, variety will unquestionably make for safety, but will not by any means assure safety, and indeed can scarcely secure the optimum result in any considerable per cent of cases. As soon as we possess an adequate knowledge of the specific properties of our natural foodstuffs and their supplementary relations to each other, it will certainly be possible to compound fairly simple and monotonous diets which can be depended upon to induce physiological well-being closely approximating the optimum.

The conscientious adherence to a vegetarian diet by one who has no adequate technical knowledge regarding the subject of diet appears to be fraught with danger since among the foods of vegetable origin, ordinarily consumed by human beings, several dietary factors are as a rule of an unsatisfactory chemical character. It is certain that all of the components of a successful diet are present in foods of plant origin.

The data which we have discussed in the present paper should we believe serve to orient investigations of this complex subject, and emphasize the great possibilities for human nutrition and animal production of properly directed research in this field.

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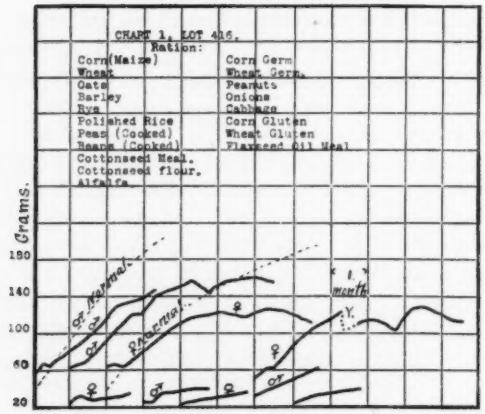

Chart 1. Lot 416 illustrates the failure of young rats to select from a liberal variety of vegetable foodstuffs the ingredients and proportions best suited to their physiological requirements. After 90 days there was no growth. One female even produced a litter of young and made an effort to rear them, but these young at the age of 90 days weighed but 35, 39, 39, 61, and 36 grams respectively. Everything necessary for complete nutrition was present in the foods regularly supplied, as is shown by the good growth of Chart 3, Lot 273, which received a monotonous mixture of corn, peas, and alfalfa flour. The cause of the poor nutrition of these animals is probably to be found in the relatively low content of proteins of poor quality, unsatisfactory relationships among the inorganic constituents of the diet, a low content of the fat soluble A and to some extent to the presence of toxic substances in certain vegetable foodstuffs. Appetite is not to be a safe-guide to the selection of food. The five short curves are those of the second generation.

In this and succeeding charts Y marks the birth of young.

This rat grew from weaning time to 83 per cent of the normal adult size on a simple mixture of polished rice 60, and alfalfa flour 40 per cent. Although somewhat undersized these rats appear nearly normal and a female has produced two litters of young after having grown up on this diet. (See legend to Chart 2, Lot 478.)

These rats were reared on a monotonous mixture of corn (maize) 50, alfalfa flour 30 and wheat germ 20 per cent. The albino is the mother of the hooded rat on the left. (See legend to Chart 4, Lot 271.)

The above photographs illustrate the appearance of three generations of rats which have been confined strictly to a diet of corn 50, alfalfa flour 30 and cooked peas 20 per cent. The mixture was finely ground so that the ingredients could not be picked out. The albino and her daughter are shown above, and her granddaughter below. (See legend to Chart 3, Lot 273.)

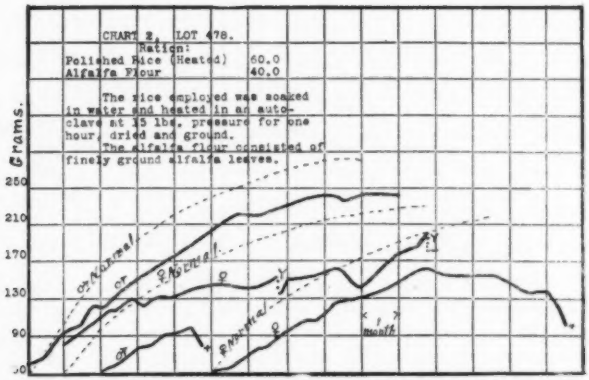

Chart 2. Lot 478 shows fairly good growth in the case of two animals to 80 and 83 per cent of the normal adult weight, on a monotonous mixture of polished rice 60 and alfalfa flour 40 per cent. These rats appear well nourished after nine months on this diet. It is evident that both the unidentified dietary factors, the fat soluble A and water soluble B are furnished by the alfalfa content of the ration, since heated polished rice contains neither of them. The cause of the failure of these rats to reach the full adult size rests principally in the low protein content of this ration, together with the relatively poor quality of the proteins. We are now studying the factors which contribute in this ration to prevent complete nutrition.

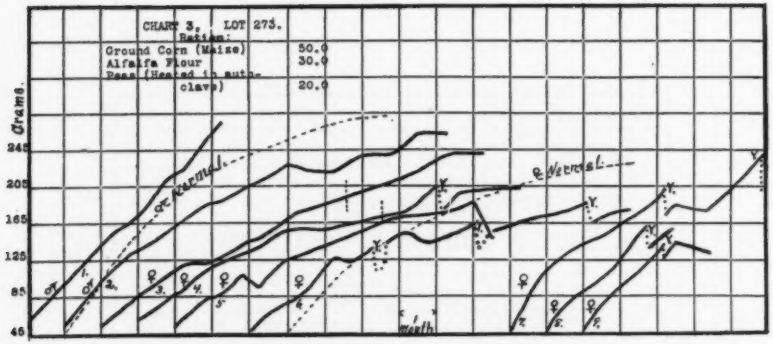

Chart 3. Lot 273 illustrates practically complete nutrition of the rat on a simple mixture entirely of vegetable origin. Rat No. 6 grew from the age of about one month on this diet of corn, alfalfa flour and peas, and produced three litters of young and two litters were successfully reared. Her daughter, No. 7, has been confined to the same diet all her life and has reached the normal adult size and has produced and successfully weaned a litter of young. These young appear perfectly well nourished and are of normal size for their age of twelve weeks.

The favorable results obtained with this ration are of special interest when considered together with the poor results of feeding a wide variety of vegetable foods, which included the three components of this ration. (Compare Chart 1, Lot 416.)

With an adequate knowledge of the dietary properties of each of the naturally occurring foodstuffs perfect nutrition can be attained in the omnivorous rat with a diet wholly of plant origin, but the animal cannot select with its appetite as a guide, the proper amounts and combinations which would best promote its well-being. Rats 3 and 4 were inferior breeders, and were isolated at the point marked on the curves and were given butter fat. There is no evidence that this improved their nutrition.

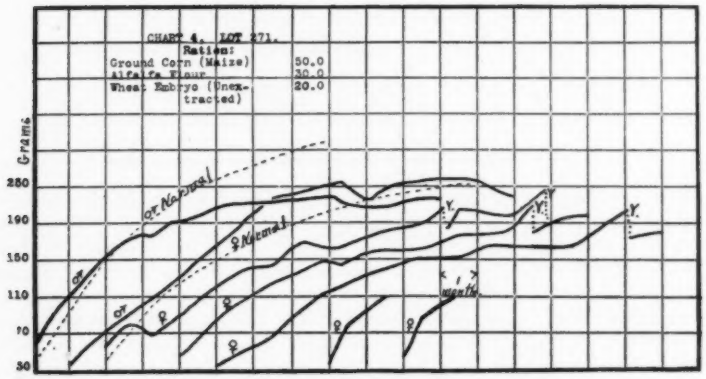
CHART 4. LOT 271.
Ration:
Ground Corn (Maize) 50.0
Alfalfa Flour 30.0
Wheat Embryo (Unextracted) 20.0
Grams
250
190
150
110
70
30
♂ Normal
♀ Normal
♂
♀
Y
Y
Y

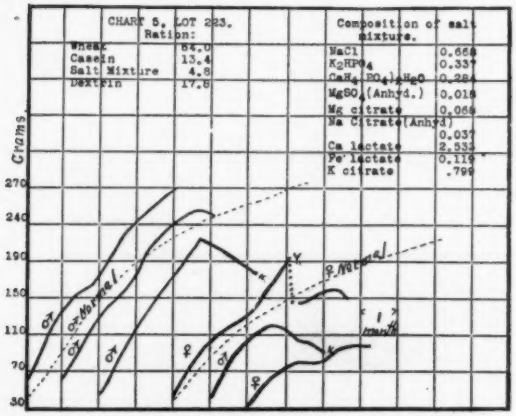

Chart 5. Lot 223 illustrates a diet which induces rapid growth for three to four months, but will not prevent rapid decline after that time. These rats presented a very miserable condition at the end of the period shown by their curves. The cause of failure is the lack of a single dietary essential, the fat soluble A. When this is supplied by the addition of butter fat the ration becomes entirely satisfactory for the completion of growth and for the normal amount of reproduction. (Compare Chart 8, Lot 223B.)

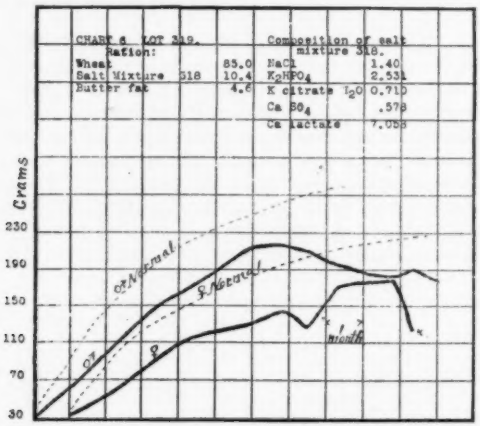

Chart 6. Lot 319 is another illustration of a ration which fails to support growth at the maximum rate and in which but a single dietary factor is responsible. (Compare Chart 5, Lot 223.) When pure protein (casein) is added to this food mixture, it becomes adequate for practically perfect nutrition of the rats. (Compare Chart 8. Lot 223B.)

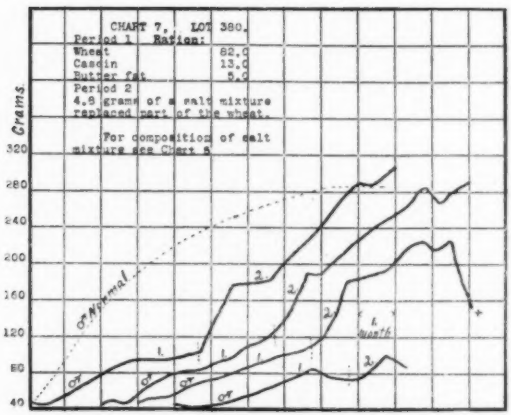

Chart 7. Lot 380 illustrates failure to grow normally on a diet which is adequate except in the character of its inorganic content. At the points marked on the curves a suitable salt mixture was added. The rats at this time had lost most of their hair and were suffering from an eczematous condition of the skin, ears, and tail. On the addition of the salt mixture there was a prompt acceleration of growth and a complete recovery from the pathological condition described. This ration with the salt mixture added is the same as that of Chart 8, Lot 223B.

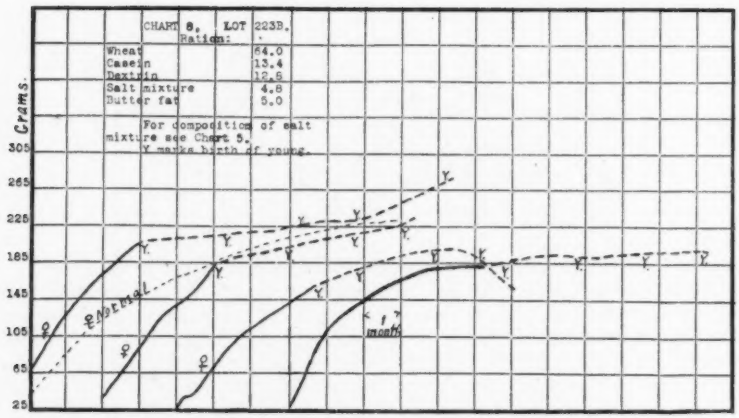

Chart 8. Lot 223B should be considered in connection with Chart 5, Lot 223, which lead to failure because of the lack of an adequate content of the fat soluble A, and with Chart 6, Lot 319, which led to failure because of a low protein content of poor quality. The above Chart (Chart 8) shows the complete nutrition of rats fed a closely similar ration with the above mentioned shortages made good. Although wheat contains a certain amount of a toxic constituent the ration of Lot 223B is otherwise so satisfactorily made up that the injurious effect of this is not apparent. (Compare Chart 9, Lot 397, Chart 10, Lot 521, Chart 11, Lot 585, and Chart 12, Lot 495.) This shows the fallacy of attempting to prove the presence or absence of an injurious character of moderate intensity in a food or drug by including it in a diet otherwise satisfactory. With a diet less efficient in one or more other factors the injury due to the toxic factor in question would become apparent.

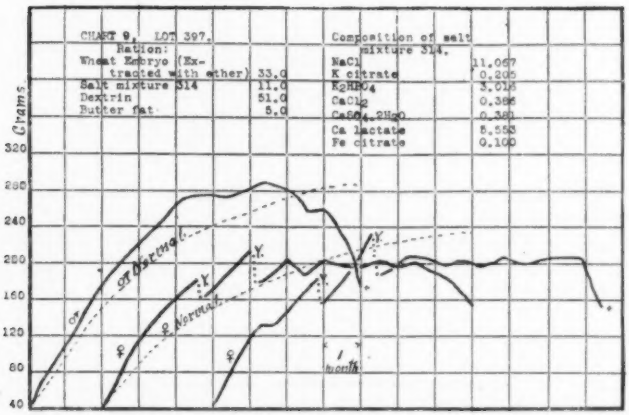

Chart 9. Lot 397 shows growth at the maximum rate on a ration of fat free wheat embryo, a salt mixture, dextrin and butter fat. Until they are nearly ten months old these rats appeared perfectly nourished. This Chart should be considered in comparison with Chart 10, Lot 521, which illustrates failure to grow normally on a ration like that of Lot 397 except that the butter fat as omitted, and with Chart 11, Lot 585, which was closely similar to that of Lot 397, except that in period 1 no salt additions were made. Chart 12, Lot 495, illustrates the injurious effect of wheat embryo which has not been freed from oil by extraction with ether. This series serves to emphasize that failure of nutrition may be induced by several factors other than by "lack of vitamines" an explanation which has in recent years seemed to satisfy both the uninitiated public and a considerable number of trained investigators.

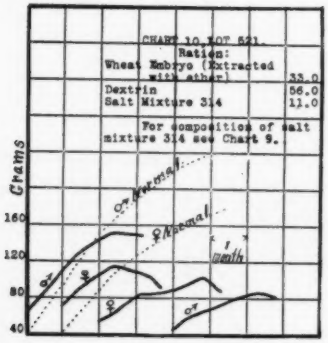

Chart 10. Lot 521. The ration of this group of rats was closely similar to that of Chart 9, Lot 397, except that the butter fat was omitted. Wheat embryo contains some of the fat soluble A, the factor supplied by butter fat, but without further additions of it this ration leads to suspension of growth and early failure. We have discussed elsewhere the dietary deficiencies of wheat embryo (11).

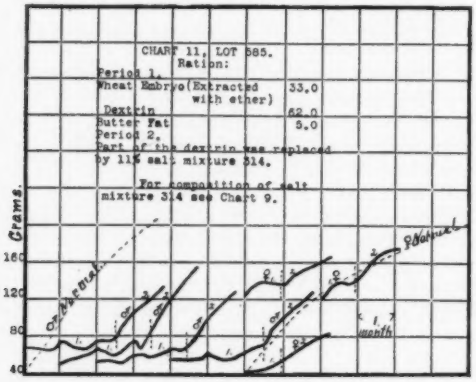

Chart 11. Lot 585 shows complete failure of rats to grow on a ration which differed from that of Chart 9, Lot 397, only in that the salt mixture was omitted. This serves as an illustration of a diet in which the omission or inclusion of a salt mixture determines complete failure or complete success in inducing growth.

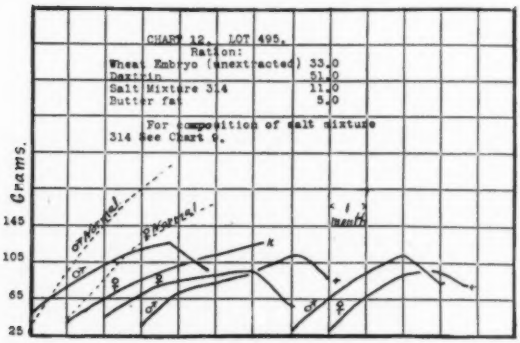

Chart 12. Lot 495 illustrates the marked injurious effect of the fats of the wheat embryo when these are included in the diet, as compared with a ration exactly similar except that the wheat embryo was extracted with ether. (Compare Chart 9, Lot 397.)

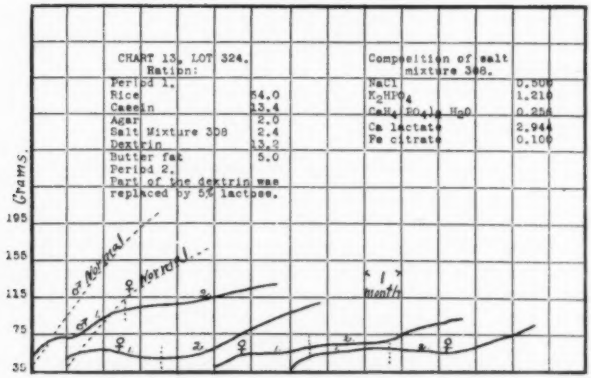

Chart 13. Lot 324 shows the failure of rats to grow on a food mixture which is lacking in but a single factor, the water soluble B. During period 2 a very small amount of this substance was added to the diet as an impurity of the 5 per cent of lactose which the diet contained. This Chart should be considered together with the records of Chart 14, Lot 401, whose diet was closely similar except that the water soluble B was added in the form of a water extract of wheat embryo.

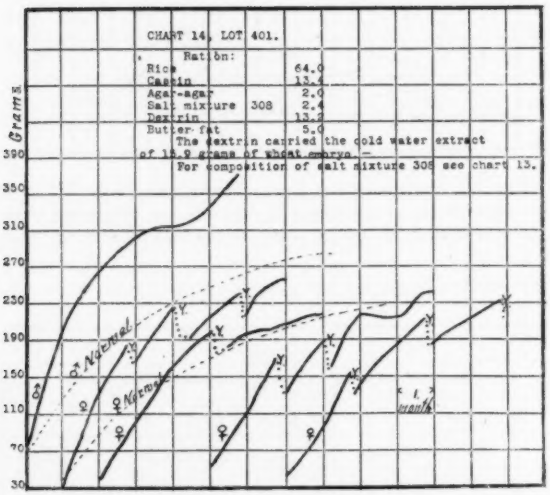

Chart 14. Lot 401, illustrates how the inclusion of the cold water extract of 15.9 grams of wheat embryo per hundred of ration transforms the food mixture from one which does not induce growth at all (Compare Chart 13, Lot 324, Period 1), to one which induced perfectly normal growth and repeated reproduction. The ration without the butter fat is again inadequate for growth. This is shown with a ration of somewhat different composition in Charts 15 and 16, Lots 417 and 418.

It is interesting to note, however, that of the nine litters of young produced by the four females in Lot 401 but two individuals were reared. The ability to reproduce and rear the young serves as a far more delicate test of the satisfactoriness of a diet than does growth (20).

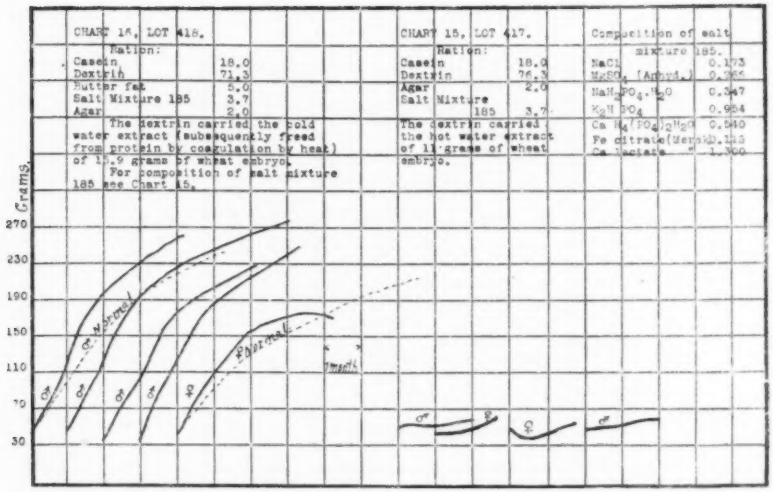

Chart 15. Lot 417 illustrates the failure of rats to grow on a ration adequate in all respects except in a lack of the fat soluble A. When the latter is supplied in the form of butter fat the food mixture supports growth at the maximum possible rate. (Compare Chart 16, Lot 418.) The contention of Funk (15) that butter fat does not contain "vitamine" (i.e., a substance indispensable from the diet) has not as yet been supported by published experimental data, secured with diets so planned as to reveal the presence of what we have termed the fat soluble A.

Chart 16. Lot 418 should be compared with Chart 15, Lot 417. The latter fails to support growth because it does not contain the unidentified dietary factor, the fat soluble A. The former which is closely similar except that this factor is supplied by the 5 per cent of butter fat contained in the food mixture supports growth at the maximum rate.

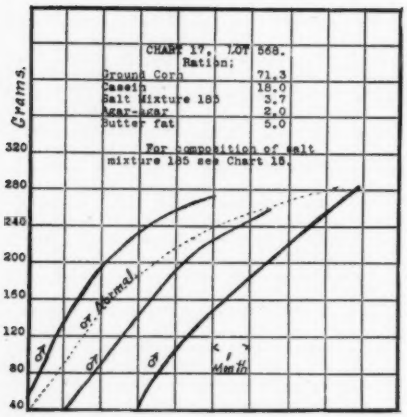

Chart 17. Lot 568 is included in the present series of illustrations to make clear the inadequacy of Voegtlin's assumption that pellagra is due to a deficiency of vitamines (17). All the chemically unidentified constituents of the diet except a part of the fat soluble A which is present in the butter fat, are supplied by the 71.3 per cent of corn in this food mixture. The failure of mice, rats, and monkeys to maintain a normal state of nutrition on a diet of corn (3) is due not to a lack of the unidentified constituents of the diet although corn is comparatively poor in the fat soluble A, but to a poor relationship among the inorganic constituents (Chart 18, Lot 618) and to its low protein content together with the poor quality of the latter. For growth in young rats the dietary A must also be increased.

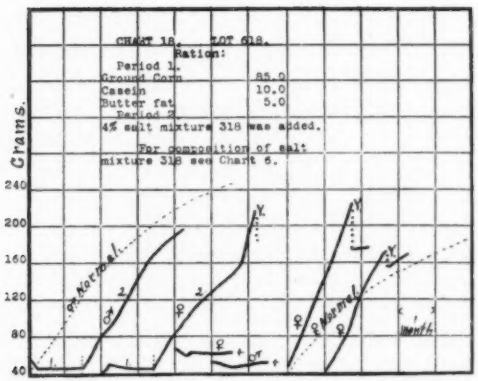

Chart 18. Lot 618 illustrates how inadequate is the character of the inorganic content of the corn kernel (maize) for growth. This ration is closely similar to that of Chart 17, Lot 568, except for a salt mixture addition in the latter. This, however, makes the difference between no growth at all and growth at the maximum possible rate. After complete suspension of growth for six weeks the addition of a salt mixture of suitable composition led to prompt resumption of growth at a rapid rate

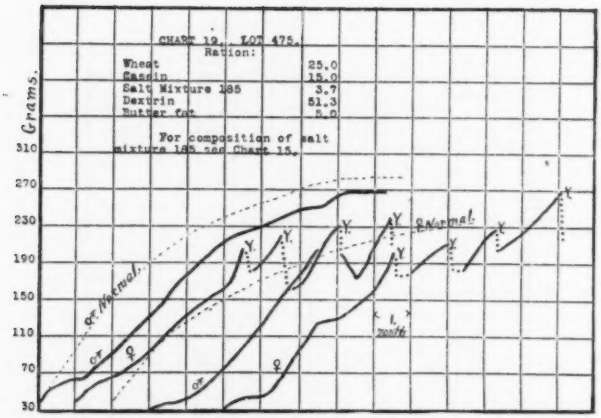

Chart 19. Lot 475 is included here to show the behavior of rats fed a ration deriving all of its water soluble B from its content of 25 per cent of wheat. This amount of wheat supplies enough of this factor to support normal growth and repeated reproduction, but does not suffice for the rearing of the young. Of the eight litters produced none were reared. This food mixture without the butter fat, is wholly inadequate to support normal nutrition in the rat over a prolonged period (Chart 5, Lot 223). While it is of course possible that there is contained in the extracts which we describe as furnishing the water soluble B more than one essential dietary factor, there is still wanting in the literature any experimental evidence in favor of the existence of more than a single indispensable chemical substance.

THE DISTRIBUTION IN PLANTS OF THE FAT SOLUBLE A, THE DIETARY ESSENTIAL OF BUTTER FAT[1]

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From the Laboratory of Agricultural Chemistry of the Wisconsin Experiment Station

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In 1913 it was pointed out in a paper from this laboratory (1) that certain fats of animal origin contained a substance the nature of which is as yet unknown, which is essential for growth or for long continued maintenance of health in the rat. Olive oil and lard were shown not to possess this growth factor. Later Osborne and Mendel described experiments which fully confirm our observations and added cod liver oil to the list of growth promoting fats, and almond oil to the list of fats which exert no special influence on growth (2). It was a matter of great importance to determine whether this indispensable dietary factor which we have termed the fat soluble A, (3) is present in foods of vegetable origin or whether it is a specific product of the mammary gland in mammals and the ovary in birds elaborated for the benefit of the suckling or of the embryo bird. We have for this reason examined various types of substances of plant origin, feeding these with rations so made up as to be wholly adequate when one of the growth promoting fats was included but inadequate when such fats were omitted. In the present paper we present the records of rats fed rations in which maize, cottonseed, linseed, olive, sunflowerseed, and soy bean oils respectively were the only source of the dietary factor supplied by butter fat.

All these have given results which make it clear that the fat soluble A is not found in fats and oils of plant origin so far as we have been able to discover. Owing to the importance of this fact, from the point of view of practical dietetics and animal production we desire to record these negative results for the more widely used plant oils.

One of the most interesting results of this investigation is the great difference in the effect on rats of consuming cottonseed oil which was prepared by extraction with ether, as compared with those fed a simi-

[1] Published with the permission of the Director of the Wisconsin Experiment Station.

lar ration but with commercial bleached oil prepared by hot pressing. The former suffered distinct intoxication within a few weeks and showed rapid loss of weight (Chart 7, Lot 512) whereas the commercial sample could be fed at the same plane of intake with no signs of injury. (Chart 7-Lot 531). This result serves to corroberate the observations of Withers and Carruth (4), that the toxic factor "Gossypol" is soluble in ether and is present in the fat prepared by the use of this solvent.

Among the other oils of plant origin which we have studied, wheat oil, linseed oil and hydrogenated cotton seed oil have shown definite but not severe toxic effects on young rats. This we have been able to demonstrate by employing as rations, with which the fats were fed, food mixtures so made up as to just admit of about normal growth for several months but so low in their content of the fat soluble A as to render the animals sensitive to any unfavorable factor which might be superimposed on it in their delicately adjusted condition. In this way we have made it possible to test for toxicity of low intensity which would never induce visible effects if present in a diet which was otherwise of good quality.

This principle should be carefully considered by those who would study the harmfulness or innocuousness of various substances which may occur in the diet (5). Any of the several factors which make up the satisfactory diet can be singled out as the one to serve as the limiting factor to be so adjusted as to lower the resistance of the experimental animals.

In experiments in which the fat soluble A of the diet was supplied wholly by vegetable products it has been found that alfalfa leaves are an excellent source of this dietary factor, and that cabbage leaves also supply it. The cereal grains, while containing a small amount, are markedly inferior to alfalfa leaves in their content of this indispensable dietary factor.

The marked success which was reported by McCollum and Davis in restoring to health by the addition of corn, rats which had been brought to the threshold of death by a diet of supposedly approximately pure foodstuffs, should now be regarded in a new light because we have since learned of the tendency of lactose, which was a constituent of the earlier diets employed by us, to carry as impurities a sufficient amount of the unidentified dietary factors, fat soluble A and water soluble B, to supplement the content of these in any natural food, to a degree not to be ignored (6). Corn kernel fed with lactose led to improvement in the rats to a degree which corn alone could not induce (6).

In the preceding paper we have described rations in which all of the fat soluble A was derived from 40 per cent alfalfa leaves, and reproduction was observed after the rats had made all their growth and had continued ten months on the diet. Corn, wheat, oats and probably other grains supply this factor but in amounts below the requirement of animals over extended periods of growth.

The superiority of the forage portion of the plant over the seed with respect to its content of the fat soluble A is of considerable interest when viewed in the light of the dietary habits of lower animals. Those which consume the forage rations grow successfully from generation to generation on a strictly vegetarian diet, while the seed eating animals, so far as we have been able to learn, normally vary their diet to a considerable degree by the addition of green leaves, worms, insects, etc. Certain combinations of seeds may however suffice for normal nutrition.

An observation of interest in the experiments reported here, is that the ether extracted residue of corn meal is more effective in causing a slow but long maintained upward trend of the curve of growth than is corn oil. Compare Chart 10, Lot 451, and Chart 3, Lot 630. The interesting point is that ether extraction of plant tissue does not remove the substances essential for growth which is contained in butter fat. The obvious working hypothesis must for the present assume that the fat soluble A is in chemical union in the plant tissues, and in a complex which is not soluble in fat or in ether. In digestion and absorption it is set free and, being readily soluble in fats, thereafter accompanies the fats in the animal body. Owing to the large content of waxes, etc., extracted from plant leaves we have not been very successful in feeding ether extracts from these sources.

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(5) Carlson, Hektoen and LeCount: Journ. Amer. Chem. Society, 1916, xxxviii, 930.
(6) McCollum and Davis: Journ. Biol. Chem., 1915, xxi, 179.
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(8) McCollum and Davis: Journ. Biol. Chem. 1915, xxi, 638 and 642.

The rat on the right hand side, a male from Lot 441, received a ration exactly like Chart I, Lot 610, page 365, except that it contained 1.5 per cent of butter fat.

The rat on the left, Lot 447, received the same ration as Lot 441 except that 5 per cent of bleached cottonseed oil replaced the 1.5 per cent of butter fat and 3.5 per cent of rice.

The special property of butter fat in contrast to the plant oil as a source of the fat soluble A is most striking.

The rat on the right, a male from Lot 441, received a ration like Chart I, Lot 610, except that it contained 1.05 per cent of butter fat.

The rat on the left, Chart 2, Lot 497, received the same ration except that 5 per cent of sunflower seed oil prepared by ether extraction replaced the butter fat and 3.5 per cent of rice.

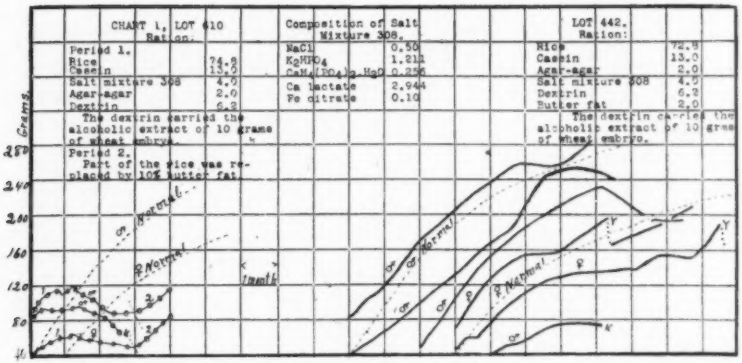

Chart 1. Lot 610 illustrates the behavior of rats fed a ration which is adequate in all respects except that it lacks one of the dietary essentials, the fat soluble A which is found in butter fat, egg yolk fat, ether extract of kidney, etc. We selected this diet as one to which the more important vegetable fats could be added in order to test for the distribution of this substance in fats of plant origin. The ration fed to this lot of rats becomes entirely adequate to support growth to maturity at the normal rate when butter fat is added. (See Chart 1, Lot 442.) There was a prompt response with growth in period 2 when butter fat was introduced.

Lot 442. The ration fed these rats differs essentially from that employed in Lot 610, Chart 1, only in containing 2 per cent of butter fat. With the butter fat present, growth is complete; without it no growth can take place. Any fat of plant origin which contains the dietary factor supplied by the butter fat, should when incorporated with this ration, induce growth.

In this and the succeeding charts Y marks the birth of young.

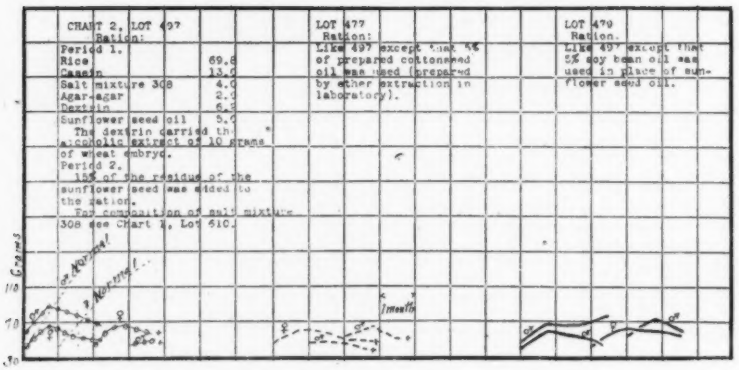

Chart 2. Lot 497 shows the failure of sunflower seed oil to promote growth when included in the ration employed in this series. With butter fat instead of sunflower seed oil normal growth would have resulted. (Compare Chart 1, Lot 442.)

Lot 477 likewise shows failure of rats fed 5 per cent of cotton seed oil prepared by ether extraction with a ration fully adequate to support growth except that it lacks the fat soluble A. These rats showed distinct signs of injury from the effects of eating even this small amount of cotton seed oil prepared by extraction in the laboratory, in contrast to commercial oil prepared by pressing and subsequent bleaching, The latter showed no toxic effects but failed to induce growth. (See Chart 4, Lot 447B.)

Lot 479 shows that soy bean oil does not have the growth promoting property possessed by certain fats of animal origin. (Compare Chart 1, Lot 442.)

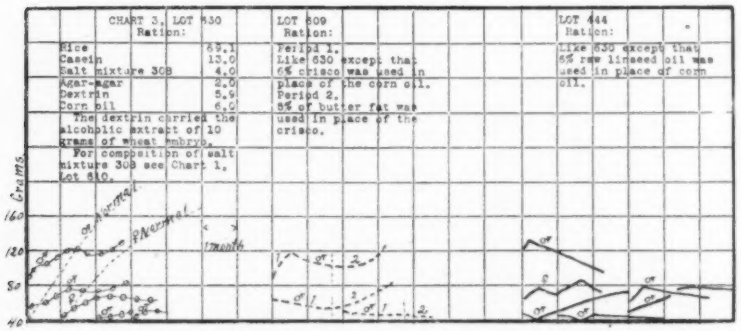

Chart 3. Lot 630 illustrates the inefficiency of corn oil to induce growth. The ration here employed is closely similar to that of Chart 1, Lot 442, except that 6 per cent of corn oil replaces 2 per cent of butter fat. The corn oil employed in this ration was prepared by ether extraction in the laboratory.

Lot 609 shows the failure of a widely used commercial substitute for lard, Crisco, made by hydrogenating cotton seed oil to induce growth in young rats. On replacing the Crisco by butter fat in the second period growth proceeded.

Lot 444, shows that 6 per cent of linseed oil fails to induce growth. We have been unable to find a single instance where the isolated fats and oils of plant origin promote growth in the manner that certain animal fats do. The rats in this group appeared very rough coated, emaciated and feeble and were in a much worse condition than those of Chart 1, Lot 610, whose ration was closely similar but contained dextrin instead of linseed oil. Linseed oil is somewhat detrimental to the rat. This does not indicate the absence of the fat soluble A from plants. We have already pointed out that alfalfa, wheat germ, maize kernel and cabbage furnish a considerable amount of this substance.

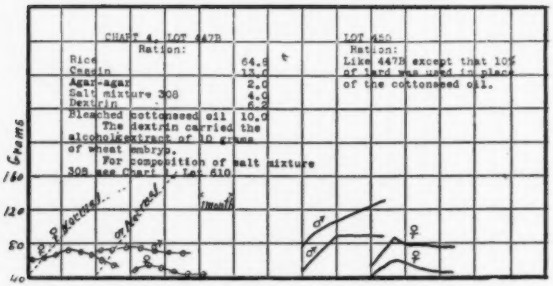

Chart 4. Lot 447B shows the absence of the dietary essential the fat soluble A from bleached commercial cotton seed oil. Even 10 per cent of the latter fails to induce appreciable growth.

Lot 450 serves to confirm our earlier observations on lard, which showed it to possess very little of the growth promoting properties of butter fat, i.e., the fat soluble A. In our early work the content of the water soluble B was dependent on its presence as an impurity in the lactose. In the present series of experiments an abundance of this factor, was added in the alcoholic extract of wheat embryo.

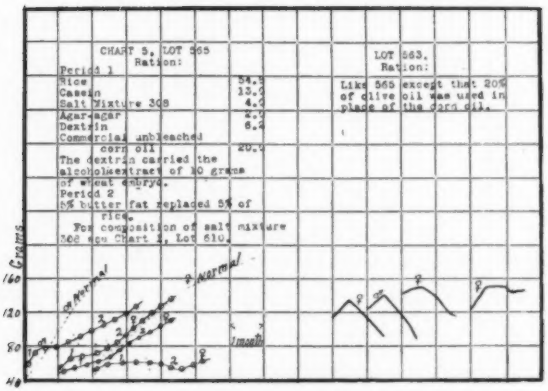

Chart 5. Lot 565. The rats in this group received 20 per cent of commercial unbleached corn oil. Even this amount does not suffice to furnish an adequate amount of the fat soluble A to induce growth.

Lot 563. In 1913 it was first shown in a paper from this laboratory that butter fat possessed a certain growth promoting property and that olive oil did not, (1) We later learned that the success of these early experiments turned upon the degree of purity of the lactose contained in the rations. The two dietary essentials, the fat soluble A and the water soluble B, at that time unappreciated, were carried by the lactose as impurities and were present in minimal amounts which could suffice to induce growth. Since pure lactose would cause failure to grow even when butter fat was included in the ration, it seemed desirable to repeat the test on olive oil with the rations known to be wholly adequate with respect to all factors except for the dietary A. The curves shown here confirm our earlier results in indicating the absence of the fat soluble A from olive oil.

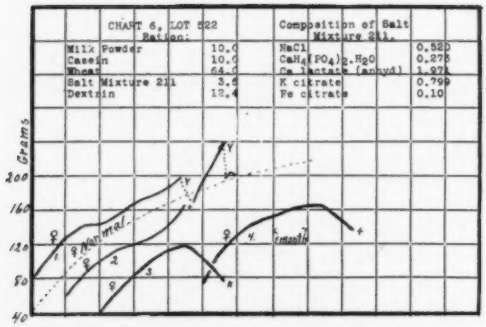

Chart 6. Lot 522, illustrates the curves of growth of four rats fed a ration which contained barely enough of the fat soluble A to induce growth at the normal rate during a period of three to five months. Number 1 produced two litters of young but did not rear any of them. After the loss of the second litter she did not appear to be in a healthy condition, so she was discarded. Number 2 died just before producing a litter of young. The remaining two suddenly declined and died after having grown fairly well for a time. With butter fat added (5 per cent) this is one of the most successful rations we have employed for inducing repeated reproduction and rearing of the young. (8)

This ration was therefore employed to test the growth promoting values of certain vegetable oils since a relatively small additional content of the fat soluble A should supplement the amount already in the ration and prevent failure. We look upon the experiments with this ration as somewhat more delicate tests for this dietary factor than were those with the ration described in Chart 1.

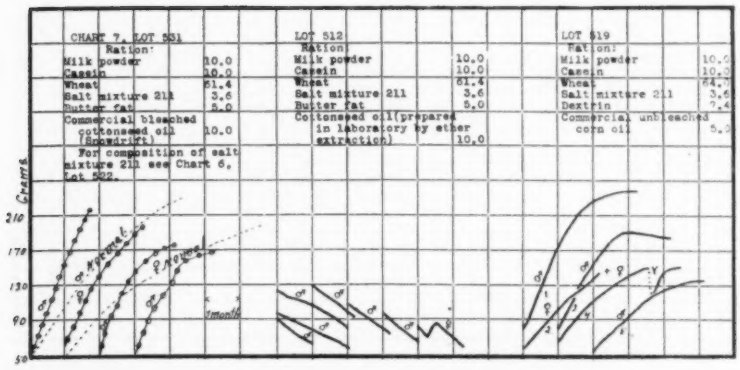

Chart 7. Lot 531. This lot was given the ration described in chart 6 but with the addition of butter fat together with 10 per cent of bleached commercial cotton seed oil to see whether the latter contained enough of a toxic constituent to interfere with growth. There is no evidence that this content of refined cotton-seed oil was detrimental. According to the manufacturers the oil was prepared by pressing.

Lot 512, shows the injurious effect of feeding the same ration as was given Lot 531 but employing instead of refined cotton seed oil a preparation made by extracting cotton seed with ether in the laboratory. This result is in harmony with those of Withers and Carruth in showing the extraction of the toxic principle of cotton seed "gossypol" by ethyl ether. (4) (Compare Chart 2, Lot 477.)

Lot 519. Received a ration like that of Chart 6 Lot 522, except that 5 per cent of commercial unbleached corn oil was included instead of its equivalent of dextrin. There was no evidence of improvement as a result of the content of corn oil. Number 2 died in parturition, number 4 produced a litter of young but destroyed them.

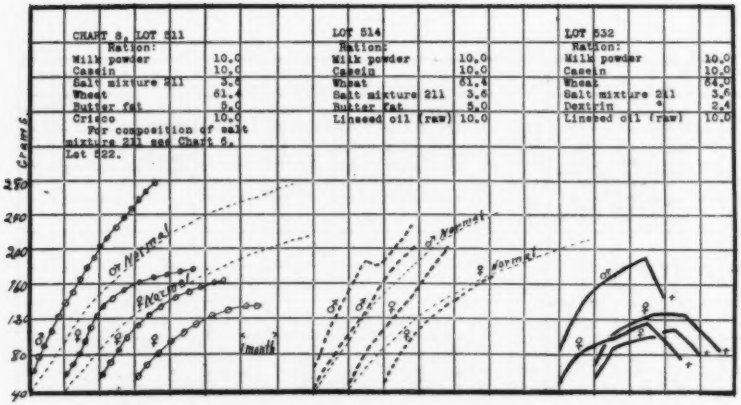

Chart 8. Lot 511. The ration of this lot was like that of Lot 522, Chart 6, but without butter fat added, and with 10 per cent of Crisco replacing an equivalent amount of dextrin. The appearance of these rats was distinctly inferior to that of Lot 522. Apparently the content of butter fat did not suffice to counteract a somewhat deleterious effect of the hydrogenated cotton seed oil. We have fed this food mixture with dextrin replacing the Crisco, and in all cases the animals were extremely sleek and well nourished (8 p. 642.) Lot 511 on the other hand suffered from roughness of the skin, tail and ears and appeared to be very poorly nourished.

Lot 514, received a ration like the preceding one but with 10 per cent of raw linseed oil replacing Crisco. There were no definite signs of any ill effects resulting from the consumption of linseed oil. Chart 3 (compare Lot 444). Linseed oil does cause injury in some degree however although its detrimental effects are not visible when the ration contains butter fat. When the butter fat was omitted from this food mixture the depressed growth and early failure of the rats was very pronounced as compared with the behavior of rats fed a similar ration but without the linseed oil. (Compare Lots 532 with 514 and Chart 6 Lot 522.)

Lot 532, shows that 10 per cent of raw linseed oil in this ration exerts a depressing effect on the growth and health of the animals. These rats became very emaciated and suffered from inflammation of the eyes. This ration with dextrin replacing the linseed oil is that feed to Lot 522, Chart 6. The bad effects of the linseed oil are not visible when the ration is improved by the inclusion of the butter fat as in Lot 514.

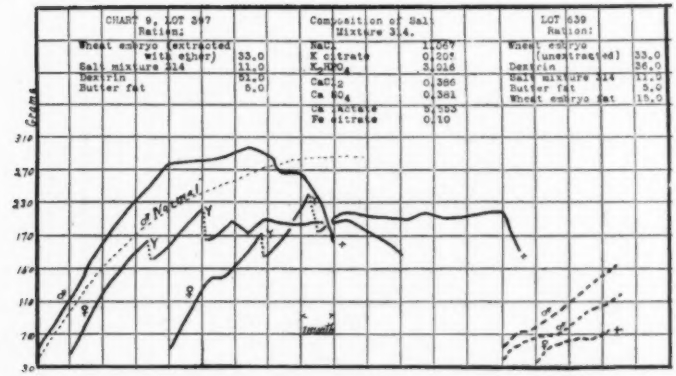

Chart 9. Lot 397 should be compared with Lot 639 whose rations were identical except that the wheat embryo in the latter was not extracted with ether and 15 per cent of the ether extract of wheat embryo was added instead of an equivalent amount of dextrin. Without the wheat embryo fats, growth progressed at the maximum possible rate. With these fats present growth was depressed to half the normal rate or less. The wheat embryo fats are distinctly toxic to animals, but this property will not be evident when fed with a highly satisfactory diet, except possibly through the accumulative effect over a long period.

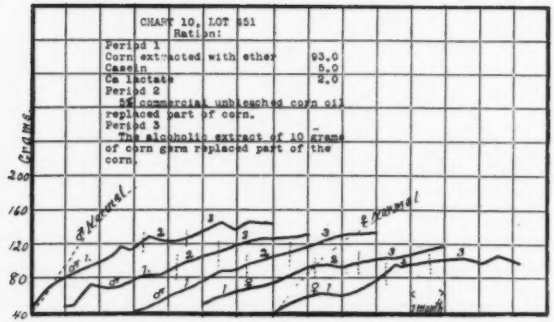

Chart 10. Lot 451, shows the property of ether extracted ground maize kernel, of promoting growth at a slow rate over a prolonged period. This, the ether extracted oil of corn, will not do. It should be noted that the inclusion of corn oil in period 2 or of the alcoholic extract of corn germ in period 3 did not produce any change in the direction of the curve of growth. The fat soluble A is present in the corn kernel but in amount below the optimum for growth, and the substance is not extracted by ether.

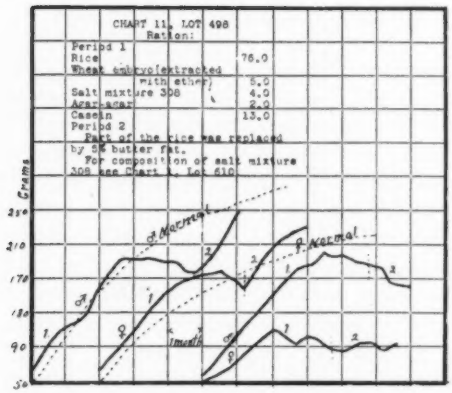

Chart 11. Lot 498. In this ration the only source of the fat soluble A was 5 per cent of completely ether extracted wheat embryo contained in the ration (period 1). During three months growth was normal but early failure ensued. That the shortage of the fat soluble A was the only factor responsible is shown by the prompt response of the two rats with growth when in period 2, 5 per cent of butter fat replaced 5 per cent of rice. There is no doubt but that this dietary factor is present in plant tissues but it seems unlikely that any isolated plant oil will contain it.

THE EFFECT OF CERTAIN PROCEDURES UPON THE COURSE OF FATIGUE IN FROG'S MUSCLE

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This research was suggested by the experiments of Lee and Salant (1), Hough and Ham (2), and Lee and Levine (3), in which attempts have been made to determine the effects of alcohol, of Ringer's fluid and of distilled water upon the course of fatigue in the excised muscle of the frog. In the experiments, as carried out by these observers, the thigh of one leg was ligated, the gastrocnemius of that leg removed and a fatigue tracing immediately made. Immediately following the application of the ligature about 2.5 cc. of water, Ringer's fluid or 10 per cent ethyl alcohol were injected subcutaneously and after allowing twenty to seventy-five minutes to elapse a tracing was made from the gastrocnemius of the other, or non-ligated leg.

In the experiments, as carried out by us, the procedure was the same as above except that in all cases the second tracing was taken from thirty to thirty-five minutes after the first, i.e., thirty to thirty-five minutes after the application of the ligature. In the cases in which a subcutaneous injection of fluid was made we used 2 cc. of Ringer's fluid or 2 cc. of 10 per cent ethyl alcohol injected into either the dorsal or anterior lymph sac.

Before the experiments had progressed very far, we noticed that in nearly every case the second, or "non-ligated" muscle did more work whether there had been a subcutaneous injection of fluid or not.

The rate of stimulation used was about thirty break induction shocks per minute, the current being interrupted mechanically and the make shocks short circuited. In a few cases, the muscles were tetanized and the amount of work done obtained in this way. In all of the experiments in which the fatigue was brought on by single contractions supramaximal shocks were used and the muscle lifted a weight of about 100 grams. In the cases in which the muscles were thrown into tetanus auxotonic contractions were used. It might be mentioned that the

TABLE 1

Relative amounts of work done by the excised gastrocnemii of frogs as shown by the total area of tracings in square centimeters

NUMBER OF EXPERIMENT	RIGHT LEG LIGATED	LEFT LEG NON-LIGATED	+ = PER CENT INCREASE IN WORK BY MUSCLE OF NON-LIGATED LEG − = PER CENT INCREASE IN WORK BY MUSCLE OF LIGATED LEG
2	108	135.3	+ 25.2
3	103.4	146.6	+ 41.7
4	109.2	114.4	+ 4.7
5	44.0	47.5	+ 7.9
6	51.1	63.0	+ 23.2
9	118.6	152.6	+ 28.6
13	100.0	154.6	+ 54.6
18	217.4	242.5	+ 11.5
39	109.7	134.4	+ 22.5
44	174.6	196.0	+ 12.2
45	96.2	110.1	+ 14.4
46	141.7	200.1	+ 41.2
66	125.7	136.0	+ 8.1
67	168.2	192.9	+ 14.6
72	64.0	105.2	+ 64.3
74	117.5	114.8	− 2.3
76	143.0	162.1	+ 10.2
78	156.5	174.0	+ 11.1
80	107.3	166.5	+ 56.6
94	98.2	107.0	+ 8.9
96	141.0	159.2	+ 12.9
98	138.0	142.8	+ 3.5
102	139.0	146.3	+ 5.25
103	102.7	146.6	+ 42.7
109	174.2	185.0	+ 6.2
113	248.3	257.7	+ 5.7
115	139.8	179.4	+ 28.3
119	84.0	134.0	+ 59.5
136	88.7	98.5	+ 11.1
137	58.4	53.5	− 9.1
138	64.3	144.3	+124.5
140	56.3	125.4	+122.7
141	87.9	115.6	+ 29.2
142	67.0	78.5	+ 17.1
143	75.4	98.0	+ 29.9
145	73.1	74.9	+ 2.4
146	35.0	72.5	+107.1
148	100.0	136.8	+ 36.8
149	120.0	129.6	+ 8.0
150	54.1	97.2	+ 79.6
151	70.1	71.5	+ 1.9
152	125.0	145.2	+ 16.1
153	94.4	141.0	+ 49.3
154	120.0	138.6	+ 15.5

TABLE 2

Relative amounts of work done by excised gastrocnemii of frogs as shown by the total area of tracings in square centimeters

NUMBER OF EXPERIMENT	LEFT LEG LIGATED	RIGHT LEG NON-LIGATED	+ = PER CENT INCREASE IN WORK BY MUSCLE OF NON-LIGATED LEG − = PER CENT INCREASE IN WORK BY MUSCLE OF LIGATED LEG
51	192.5	169.7	−13.4
52	111.7	88.0	−26.9
53	111.0	184.0	+65.7
54	166.9	173.8	+ 4.1
55	195.3	181.0	− 7.8
56	160.0	213.5	+33.4
57	166.2	215.4	+29.6
58	105.4	147.3	+39.8
59	176.3	169.8	− 3.8
60	162.4	179.9	+10.7
61	146.3	148.5	+ 1.5
63	147.6	151.2	+ 2.4
64	86.0	131.7	+53.1
65	190.3	163.7	−16.2
73	120.4	127.4	+ 5.8
75	84.0	111.0	+32.1
79	108.9	137.0	+25.7
81	205.3	200.8	− 2.2
86	88.5	120.8	+36.4
91	113.0	143.2	+26.7
95	87.8	150.2	+71.0
99	71.2	108.6	+52.5
107	180.6	199.5	+10.4
110	122.7	178.2	+45.2
114	214.9	225.6	+ 4.9
116	122.0	167.2	+37.0
117	57.5	61.3	+ 6.6
118	158.5	193.1	+21.8
123	97.3	87.0	−11.8
124	77.0	92.0	+19.4
125	66.4	115.0	+73.1
126	143.5	158.4	+10.4
128	34.0	44.0	+29.4
129	93.0	98.0	+ 5.4
130	77.5	104.0	+24.2
132	116.0	123.5	+64.6
133	98.9	136.2	+37.7
134	31.6	75.0	+137.3
135	72.2	92.0	+27.4
207	64.0	84.0	+31.2
208	38.3	43.7	+14.0
209	50.0	70.4	+40.8

TABLE 3

Relative amounts of work done by the normal gastrocnemius and by the gastrocnemius the other leg thirty to thirty-five minutes after injecting 2 cc., of 10 per cent alcohol into dorsal lymph sac

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	ALCOHOLIZED LEG LEFT LEG	+ = PER CENT INCREASE IN WORK BY ALCOHOLIZED MUSCLE − = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
8	49.6	109.7	+121.2
11	143.0	176.4	+ 23.2
15	129.4	139.0	+ 8.5
20	351.4	317.0	− 10.8
21	188.7	224.0	+ 18.7
23	211.5	219.0	+ 3.5
26	231.5	267.1	+ 15.3
32	227.4	264.5	+ 16.3
34	302.2	220.4	+ 37.1
35	159.0	214.2	+ 34.7
36	169.5	170.5	+ 0.6
48	170.5	167.5	− 1.7
69	187.6	181.7	− 3.2
82	234.4	239.4	+ 2.1
83	139.8	154.8	+ 10.6
88	90.5	117.6	+ 29.9
92	108.8	114.2	+ 4.6
100	148.0	153.5	+ 3.7
104	122.3	90.8	− 34.6
155	161.2	155.8	− 3.4
156	110.0	198.4	+ 80.3
157	172.6	154.1	− 12.0
158	185.2	208.4	+ 12.5
159	168.3	193.9	+ 15.2
160	155.4	200.8	+ 29.2
161	129.3	134.5	+ 4.0
162	115.6	150.6	+ 30.2
163	167.6	218.3	+ 30.2
164	160.7	151.3	− 6.2
165	118.3	125.4	+ 6.0
167	109.7	134.4	+ 22.4
168	161.3	180.3	+ 11.1
169	117.5	119.0	+ 1.2
170	57.3	56.6	− 1.2
171	205.9	213.1	+ 3.4
172	172.6	197.3	+ 14.2
173	188.2	186.2	− 1.0
174	160.6	192.6	+ 19.9

TABLE 3—Continued

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	ALCOHOLIZED LEG LEFT LEG	+ = PER CENT INCREASE IN WORK BY ALCOHOLIZED MUSCLE − = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
175	191.7	192.2	− 0.5
176	184.7	177.0	− 4.3
177	138.0	160.7	+ 16.4
178	122.7	124.4	+ 1.3
179	115.0	128.0	+ 11.3
180	123.7	147.7	− 11.0
12	74.5	185.1	+148.4
29	158.0	216.3	+ 36.8
33	118.3	168.5	+ 32.4
38	158.0	172.0	+ 8.8

TABLE 4

Relative amounts of work done by the normal gastrocnemius and by the gastrocnemius of the other leg thirty to thirty-five minutes after injecting 2 cc. Ringer's fluid into the dorsal lymph sac

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	AFTER RINGER'S LEFT LEG	+ = PER CENT INCREASE IN WORK DONE BY THE MUSCLE AFTER RINGER'S FLUID − = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
7	112.0	205.8	+ 83.7
10	68.7	128.0	+ 87.7
14	76.5	219.0	+186.2
17	210.9	190.4	− 10.7
22	298.3	249.8	− 19.4
24	217.0	220.3	+ 1.5
25	197.0	192.4	− 2.3
27	236.2	216.0	− 9.3
31	179.7	143.0	− 25.6
37	173.3	217.9	+ 25.7
40	162.0	150.3	− 7.7
41	147.3	152.4	+ 3.4
42	128.5	106.5	− 20.6
43	114.5	103.0	− 11.1
47	153.7	166.0	+ 8.0
50	152.7	118.4	− 28.1
70	95.0	122.9	+ 29.3
85	119.2	143.7	+ 20.6
89	107.5	123.3	+ 14.7

TABLE 4—Continued

NUMBER OF EXPERIMENT	NORMAL LEG RIGHT LEG	AFTER RINGER'S LEFT LEG	+ = PER CENT INCREASE IN WORK DONE BY THE MUSCLE AFTER RINGER'S FLUID − = PER CENT INCREASE IN WORK BY NORMAL MUSCLE
93	123.4	111.0	− 11.1
97	110.2	150.8	+ 50.4
101	198.4	153.7	− 22.5
105	108.3	125.0	+ 15.4
182	119.4	140.4	+ 17.6
183	155.3	151.5	− 2.5
185	148.0	192.9	+ 30.3
186	127.8	147.0	+ 15.0
187	82.4	98.5	+ 19.5
189	136.7	123.0	− 11.1
190	113.0	127.5	+ 12.8
191	122.5	130.1	+ 6.1
192	128.9	126.0	− 2.4
193	126.4	131.5	+ 4.0
194	126.7	151.1	19.2
195	172.3	170.1	− 1.3
196	133.0	136.0	+ 2.2
197	144.7	150.0	+ 3.6
198	145.4	167.7	+ 15.3
199	157.5	166.4	+ 5.6
200	201.4	206.5	+ 2.5
201	172.0	171.1	− 0.5
202	236.7	240.4	+ 1.5
203	121.0	171.6	+ 41.8
204	170.5	208.1	+ 22.0
205	191.5	168.3	− 13.7
206	140.5	189.1	+ 34.5

two methods gave practically the same results so far as the amount of work done was concerned. The total amount of work done was determined by measuring the total area of the tracings with the planimeter as suggested by Hough and Ham (2).

The above tables give a summary of the experiments done.

RESULTS

In table 1, we give the per cent increase or decrease of work done by the muscle of the left leg which was the second, or non-ligated leg. In this table in which there are 44 experiments the average per cent of

increase by the "non-ligated" muscle is 28.76. In this table, it will be noticed that in only two cases did the muscle of the second, or non-ligated leg, fall below that of the first or ligated leg.

In table 2, the right leg is the non-ligated leg. In this table, in which there are 42 experiments, the per cent of increase of the right muscle, which is the muscle of the non-ligated leg, is 25.22. In this table in only seven cases did the muscle of the second leg fall below that of the first.

In table 3, in which 48 experiments were carried out, the per cent of increase by the non-ligated, or "alcoholized" muscle is 16.84. In this table there are nine cases in which the amount of work done by the alcoholized muscle is less than that by the normal muscle. In all but four cases we used the left leg for the alcoholized leg.

In table 4, in which 46 experiments were carried out, the per cent of increase by the muscle of the second leg following the absorption of Ringer's fluid is 12.61. In this case, it will be noticed that there are sixteen experiments in which the second muscle did less work than the muscle of the normal leg.

We found in about two-thirds of the cases of the alcoholized muscle that the initial contraction is slightly less in height than that of the normal muscle; however, the same is found to be true in the second muscle after the injection of Ringer's fluid or even after the other leg has been ligated for thirty minutes and no fluid injected. The phenomenon of introductory contractions was present in only a few cases.

The "stair-case" is more gradual and the maximum is reached later in the muscle of the non-ligated leg than in the normal muscle whether there has been a subcutaneous injection of fluid or not. The height of contractions and the number of individual contractions is, in the majority of cases, greater in the muscle of the second, or non-ligated leg. The oncoming of fatigue and the appearance of exhaustion is delayed in the majority of the cases in the muscle of the non-ligated leg. In other words, the results heretofore claimed for alcohol and Ringer's fluid are found to be present in the case of the muscle from the second leg whether there has been a subcutaneous injection of fluid of not. All of these results are as marked in the cases of comparison as in the cases following alcohol or Ringer's fluid; the increase in the amount of work done by the second muscle is greater when there is no injection of alcohol or Ringer's fluid.

It was noticed, as reported by Lee and Salant, that the increase in amount of work done by the second muscle is greater in the case

of winter frogs. This is true in the cases of comparison as well as following alcohol or Ringer's fluid. We did not find contracture more pronounced in the normal muscle. The early appearance of contracture we found depended upon the strength of the stimulus used. In those cases in which we tried to use a supra-second-maximal stimulus, we found that the muscle went into an early and marked contracture. In the cases in which the stimulus was not above the second maximum, there was no early contracture. In a few cases there was a delayed contracture, even with the rate of stimulation of 30 per minute.

CONCLUSIONS

1. The ligation of one leg increases the working power of the excised muscle of the other leg after a wait of thirty minutes.

2. That, while the alcoholized muscle or the muscle of the leg following the absorption of Ringer's fluid does more work than the normal muscle, it does this work, not on account of the effect of the alcohol or Ringer's fluid, but as the result of the ligation of the other leg.

3. Both alcohol and Ringer's fluid have reduced the working power of the muscle.

4. The method described cannot be used to determine the effect of drugs on the working power of an excised muscle.

5. It is suggested that the ligation of one leg may bring about an increase in the working power of the muscle of the other leg by reflexly causing an increase in blood pressure and thus improving the circulation through the muscle of the non-ligated leg during the thirty minutes wait. It may be that the effect noticed is partly due to secretion of adrenalin following the operative procedure. These are simply suggested as possible explanations of the results noticed.

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THE BLOOD PRESSURE IN EXPERIMENTAL PNEUMONIA

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In 1914 Newburgh and Minot (1) made numerous observations of the blood pressures in pneumonia in the wards of the Massachusetts General Hospital, to which were added thirty cases from the Johns Hopkins Hospital. It was found that the average of readings taken morning and afternoon during the course of the disease was from 110 to 120 mm. Hg. The normal for human beings of this age is 120 to 130 mm. In the fifteen cases observed in the Massachusetts General Hospital the lowest pressure was 90 mm. There were three cases at 100 mm.

These clinical studies were made to determine the prognostic value of the blood pressure in pneumonia. It was felt at the time that since the pressures did not fall during the course of the disease, nothing would be gained as an indication for treatment by recording the pressure two or three hours before death. It would then be too late for therapeutic measures. The teaching at that time was that persons who were to die of pneumonia would have a low or falling pressure days before the fatal issue.

In the course of our studies in experimental pneumonia we frequently observed that the carotid artery seemed to contain less blood than usual, a few hours before death. It was therefore of interest to measure the blood pressure in experimental pneumonia (2), and especially to follow it to the end.

We present in table 1 the results of our observations. The measurements above the horizontal line are from the curves recorded in the work upon "The State of The Vasomotor Apparatus in Pneumonia," (3) those below the horizontal line were made for this present paper, especially to determine the pressure just preceding death.

It will be observed in table 1 that while the pressures are somewhat lower than normal, there is but one case in which the fall was such as to endanger life; and this dog, No. 12, was measured literally at the

TABLE 1

The blood pressure in experimental pneumonia

ANIMAL	NUMBER	TEMPERATURE	BLOOD PRESSURE		TIME BEFORE DEATH	REMARKS
			Diastolic	Systolic		
Cat.....	2	38°C.		105	6 hrs.	
Cat.....	4	41		85	3 hrs.	
Cat.....	11	40		210	5 min.	Death due to ether.
Cat.....	13	34		150	2 hrs.	
Dog.....	8	40		135	3 hrs.	Experiment was ended by killing animal.
Dog.....	12	34		30	2 min.	At point of death when pressure was measured.
Dog.....	13	40		100	2 hrs.	
Rabbit...	51	40		82	2 hrs.	
Dog.....	109	36	85		25 min.	
Dog.....	110	36	85	105	15 min.	
Dog.....	111	39.5	75	100	40 min.	
Dog.....	112	40.5	50	85	1 hr.	
Dog.....	113	39.5	45	70	25 min.	
Dog.....	114	37	60	85	45 min.	
Dog.....	115	38.3	45	85	70 min.	
Dog.....	116	39.3	60	90	5 hrs.	Two measurements from same dog.
Dog.....	116	39	48	80	2 hrs.	

TABLE 2

The blood pressure in pneumococcus septicaemia

ANIMAL	NUMBER	TEMPERATURE	SYSTOLIC BLOOD PRESSURE	HOURS BEFORE DEATH
Rabbit.....	2	41	100	3½
Rabbit.....	3	40	102	3
Rabbit.....	6	40	80	2
Rabbit.....	9	40	85	1
Rabbit.....	10	40	75	2
Rabbit.....	13	41	100	1½

point of death, which took place not more than two minutes after the record. On the other hand, in the experiment of April 7, the dog had a pressure of 105 mm. fifteen minutes before his death.

It will be interesting to compare the records of table 1 with those of table 2 in which the pneumococcus septicaemia was produced in six rabbits by injecting the organism into the vein. This procedure is

followed by the same bacteraemia observed in fatal cases of pneumonia but without any inflammation of the lungs. In these cases the pressure is very satisfactory even two hours before death.

CONCLUSION

The blood pressure in experimental pneumonia is frequently somewhat less than normal, but, in our experience, has never been so low as to endanger life.

REFERENCES

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

I. The Occurrence and Measurement of a Sudden Change in the Rate of Growth of Avian Ova

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The physiology of reproduction has always been of interest and importance to the practical breeder. It is only recently however that this branch of physiology has shown itself to be so intimately connected with important and varied functions of the organism as to command much attention from physiologists generally. More recently still it is becoming clear that further advance in unravelling the complicated processes of heredity is, in part at least, dependent upon a closer knowledge of the *exact conditions* under which the germinal elements form, grow, function, mature, unite, and take the first steps of embryonic development.

Our choice of material has been determined by two circumstances which combine to make it seem specially desirable at this time to amplify at several points our present information on the physiology of reproduction in birds. In the first place, very extensive studies involving perhaps the greatest amount of control of hereditary processes yet attained have been carried out upon these forms[1] by Whitman, and later by myself (1, 2, 3); furthermore, new and exact data on the storage metabolism of the ova of pigeons—the identical material used by Whitman—have now demonstrated (3, and unpublished data) not only a correlation of this metabolism with the sex of the offspring, but a fact that necessarily follows, namely, that this high or low degree of storage in the ova—and thus the sex—cannot be wholly independent of the metabolism of the organism. In the second place, the ovary, and many of the individual ova, of the bird are large enough to permit

[1] Only a fraction of the data have as yet been published.

of individual study, and of methods of study hardly available elsewhere among animals.

It happens therefore that avian reproduction supplies a gateway through which some of the results and methods of modern physiology may be brought to bear upon a fundamental problem of heredity and of sex.

Primary oöcytes are known to be already formed in a female chick one week old, and in several species from widely separated groups of birds it is known that the oögonia are formed at a correspondingly early period. It is well known too that a "growth period" marked by the deposition of yolk begins at once. In general this growth continues until the maturation of the egg, and its extrusion from the ovary.

It is also generally understood[2] that the rate of growth is not uniform, or continuous, and may be divided into a number of periods. In a previous study (7) I found some evidence that the growth period in the fowl is not a steady, unbroken one—not even among the larger continuously growing ova of a functioning ovary; but at a certain point the rate quite suddenly changes to a rate apparently eight to twenty times faster than before; and further, that the change in rate normally occurs five to eight days before ovulation, and when the ova have reached a diameter of about 6 mm.

It seemed desirable further to study and to test these indications since if such a radical change in growth really occurs it is probably a fact of importance in the origin of the germ cells; furthermore, the changes seemed to occur at a stage when the egg is large enough (6 mm.) to be available not only for the usual cytological study, but for macroscopical observation and physiological experiment as well.

The earlier evidence indicative of such change in the growth-rate was of two kinds: First, ova of the fowl which were less than 6 mm. in diameter failed to take up Sudan III from the blood stream;[3] while growing ova of more than 6 mm. in diameter readily took up the dye. The second kind of evidence was obtained by a comparison of the thickness of striations found in one small ovum (7 mm.) with other striations generally present in larger ova. We quote as follows from our former paper (l.c., p. 460):

[2] See D'Hollander (4), Sonnenbrodt, (5), Bartelmez (6) and others.

[3] I.e., not in amounts macroscopically observable. I have elsewhere (7) described in detail the use of Sudan in the measurement of the rate of growth of ova.

Here (in the 7 mm. ovum) the noteworthy facts are, that a striation exists, and that the lamellae are not thicker than 0.25 mm. Whether these lamellae are made up of still smaller strata which really represent days of growth I am quite unable to say. I doubt somewhat that the radius of these small eggs is increased by as much as 0.25 mm. in twenty-four hours; anyway these strata offer some evidence—in the light of what we know of succeeding yolk strata—that these small eggs do not grow *faster* than 0.25 mm. per day (the larger eggs grow at the rate of 2 mm. per day).

From indications then at hand it seemed pertinent (p. 460) to ask

What is the meaning of the extraordinary difference in growth-rate of eggs under, and over, 6 mm. in diameter? What old mechanism is inhibited, or what new one brought into action, that will account for this procession of cells—each with *months* of slow and constant growth behind it—coming to a point from which it jumps in a *day* from its accustomed rate of increase, to a rate that is probably from eight to twenty times higher? Do the follicular cells now become more permeable than formerly to the ingredients of yolk? Is the increased vascularity of the follicular envelopes, that certainly occurs at this time, a cause or a result of the new activity?

In considering methods of obtaining positive and crucial evidence on the question of the existence and amount of such difference in growth-rate it was seen that if the eggs of more than 6 mm. diameter grow twenty times as fast as the eggs of smaller diameter, then the number of eggs above and below 6 mm. in a functioning ovary should, at any time, bear a 1 : 20 ratio to each other. That is, there should be twenty times as many ova under 6 mm. *included in a given unit of radius*—say 1 mm. or 3 mm.—as there are ova of more than 6 mm. within the same unit of radius. Or, concretely, there should be twenty times as many eggs between 3 and 6 mm. in diameter in a ovary as there are eggs between 6 and 9 mm. in diameter in the same ovary. It follows therefore that one can thus count and classify the ova in a number of appropriate ovaries, and then *calculate* the difference in rate of growth in the two groups of ova.

In actual practice all the ova above 6 mm. diameter have been counted and this total number compared with the number of eggs of 3 to 6 mm. diameter in the same ovary. Considering the final size of the eggs to be 30 mm., the rapid growth period involves the addition of 24 mm. From these data the relative rate of growth is obtained from two simple proportions.[4]

[4] E.g., if (as in ovary No. 1) there are 5 of the larger ova (= 24 mm. growth) and 19 smaller ones (= 3 mm. growth), we find, 3 : 19 : : 24 : x; x = 152; then 5 : 152 : : 1 : x; here x = 30.4 the number sought.

For this study were used only fresh ovaries known to be actually discharging ova—as shown by egg laying record, by eggs in oviducts, or by freshly opened follicles. Most of the material was obtained from a butcher near by who permitted the removal of the ovaries from the birds as soon as they were killed. Eight of those studied and tabulated here were our own birds, whose egg-laying history was known. During two summers nearly two hundred ovaries were examined; complete and exact measurements were however made on only forty of these.

The result as determined upon this material by this method of calculation is subject to error from the following sources: (1) A functional ovary from an old bird may be nearly approaching the extrusion of the last ovum this bird can ovulate; in this case the calculated rate of growth may be much too low (see Nos. 4–23–27–30 in table 1). (2) The largest ovum may have escaped from the ovary but a few hours before death. These few hours might bring no change in the number of ova measuring 3 to 6 mm., but would change the number above 6 mm. by one-sixth of the total. This is probably the true history of nearly half of the ovaries here tabulated (since only ovaries from laying hens are considered), and tends to make the calculated result too high. It has therefore been thought wise to base the calculation upon the actual number found, and then upon this actual number plus one (see column 6, table 1). The actual rate more probably lies between these two figures. (3) The occurrence of follicular atresia, or the resorption of ova, may modify the result in either direction; particularly if the larger resorbed ova become confused with degenerating capsules from which eggs have been liberated. Where such conditions were confusingly in evidence we have rejected the material. (4) In a few cases it has been found difficult to know whether the largest of the slow-growing eggs has yet begun to deposit yellow yolk—that is, whether it has already started the new rate of growth; and in two cases (see Nos. 17 and 21, table 1) there were two and three respectively of such ova of doubtful classification. Error in making this classification may modify the result in either direction. (5) The final diameter of the yolk of the fowl is not uniformly 30 mm.; probably it is oftener more than less. If so, the actual sudden increase in rate of growth of ova when they reach 6 mm. diameter is somewhat greater than is indicated by our figures.

A diameter of 6.0 mm. was not always and arbitrarily taken as the dividing point, but in each ovary this point was determined by the diameter of the largest ovum with no yellow yolk evident at its surface. If this proved to be 6.5 mm. the number of ova between this and 3.5 mm. was determined and used in the classification.

The data of tables 1, 2 and 3 seem to establish beyond question the fact of a sudden increase in the growth-rate of ova of the fowl when these attain a diameter of about 6 mm. It is further shown that the extent of this increase is somewhat greater than our earlier evidence

TABLE I

I		II	III	IV	V	VI
June	14	1	5	19	30.4	25.3
	14	2	8	17	17.0	15.1
	14	3	7	19	21.7	19.0
	14	4	4	3	6.0	4.8
	14	5	6	21	28.0	24.0
	14	6	6	27	36.0	30.9
July	3	7	5	20	32.0	26.7
August	3	8	5	28	44.8	37.3
	3	9	6	27	36.0	30.9
	3	10	7	19	21.7	19.0
	17	11	5	24	38.4	32.0
	17	12	6	20	26.7	22.9
	22	13	6	20	26.7	22.9
	22	14	4	19	38.0	30.4
	22	15	5	32	51.1	42.6
	24	16	4	12	24.0	19.2
	24	17	4 (2)	8	20.0 16.0 10.7	18.0 14.5 9.9
	24	18	4	17	34.0	27.2
	24	19	5	12	19.2	16.0
	28	20	6	14	18.7	16.0
	28	21	5 (3)	21	38.4 33.6 21.0	32.0 28.0 18.7
	31	22	6	22	29.3	25.1
	31	23	4	10	20.0	16.0
September	7	24	5	17	27.2	22.7
	7	25	4	20	40.0	32.0
	7	26	7	16	18.3	16.0
	7	27	6	8	10.7	9.1
	14	28	7	14	16.0	14.0
	14	29	5	15	24.0	20.0
	21	30	5	9	14.4	12.0
	21	31	4	21	42.0	33.6
		Av. =5.4		18.9	28.0	23.6

The several columns bear the following data: I = date of examination of ovary; II = serial number of ovary; III = the number of ova more than 6.0 mm. diameter in ovary; IV = number of ova between 3.0 and 6.0 (approximately) mm.; V = calculated difference in rate of growth of the two groups of ova; VI = this difference of rate when (1) is added to the number of larger ova actually found (see text). Average does not include Nos. 4–17–21–27. The dates refer to 1911 and 1912.

had suggested; the present data indicating the rate to be between 23.6 and 28 times more rapid in the larger ova. The average of these two numbers, i.e., 25.8, probably very nearly measures the remarkable extent of this sudden jump in growth power.

That the whole of this saltation in rate of growth may be completed in as short a space of time as a day is shown by an examination of the data presented in tables 2 and 3 taken in connection with the following considerations: (1) The commonly observed fact that one of the largest ova may be ovulated daily. (2) In such cases an egg of the 6 mm. group must daily join the restricted group of the larger ova. Now, the size differences of the very restricted number of eggs immediately over 6 mm. in diameter indicate (tables 2 and 3) that the full extent of the new growth power is often attained in a single day.[5] Convincing evidence of another kind on this point resulted from a direct measurement (7, p. 459) with our Sudan III method of studying the rate of growth of the more central and peripheral yolk of the hen's ovum. The indicated rates of growth were 1.53 mm. for the more central[6] portions, and 1.67 mm. for the peripheral portions of yolk per twenty-four hours. The difference here is within the limits of error of measurement. The first yolk that is added to an ovum already 6.0 mm. in diameter is therefore added at about twenty-five times the previous rate.

It is of some interest to note the part that seasons play in our data on the indicated increase in rate of growth. This rate seems higher in the early summer: 27.4 = average of first ten ovaries; and lower in late summer: 21.6 = average of last ten ovaries. It is doubtful however whether importance attaches to these figures.

The change in the rate of deposition of yolk involves the follicular membrane—which everywhere covers the surface of the ovum—in a way that is interesting to note. The radius of the ovum is increased in the period of rapid growth by nearly 2.0 mm. in twenty-four hours. This requires, during each thirty minutes or less, the passage through (or between) each of these follicular cells of a quantity of yolk material equal to the volume of the cell itself. The material thus transported

[5] Ovaries No. 2–6–8–17–26 on the other hand show two of these ova of nearly the same size, and No. 21 contained 3 such ova of nearly equal size. But these constitute only one-fifth of the total and we know that hens sometimes lay two eggs within less than twenty-four hours, and that they also sometimes lay double-yolked eggs. Follicular atresia too may eliminate some.

[6] I.e., parts immediately *external* to the 6 mm. central sphere of white yolk.

TABLE 2

Showing detailed measurements, in millimeters, on eight ovaries (early summer) tabulated in table 1. The line separates eggs growing at vastly different rates

NO. 1	NO. 2	NO. 3	NO. 4
32 × 34	28 × 30	28 × 33	32 × 35
30 × 33	25 × 30	26 × 28	24 × 27
25 × 30 = 5	24 × 27 = 8	25 × 27 = 7	22 × 23 = 4
13 × 15	20 × 22*	23 × 24	16 × 18
9 × 10	13 × 17	21 × 25	5 × 5
6 × 6	10 × 18	16 × 18	3½ × 4 = 3
5 × 6 = 3†	8 × 8	6 × 6½	3 × 3
4½ × 5	6½ × 7	5½ × 6 = 2	
4 × 5 = 19	6 × 6 = 3	5 × 5	
4 × 4 = 4	5½ × 6	4½ × 5 = 2	
3½ × 4 = 2	5 × 5 = 3 = 17	4 × 4 = 3 = 19	
3 × 4	4½ × 5 = 2	3½ × 4 = 4	
3 × 3 = 6	3 × 4 = 2	3 × 3½ = 4	
	3 × 3½ = 2	3 × 3 = 3	
	3 × 3 = 4		

NO. 5	NO. 6	NO. 7	NO. 8
25 × 29	23 × 27	28 × 33	28 × 30
23 × 26	22 × 28	26 × 29	21 × 25
19 × 20 = 6	15 × 18 = 6	20 × 23 = 5	12 × 14 = 5
18 × 19*	13 × 14	14 × 17	7 × 8
15 × 18	8 × 8½	8 × 11	6 × 7
7 × 7½	6½ × 8	6 × 6	6 × 6
6 × 6 = 2	6 × 6	5½ × 6	5½ × 6
5½ × 6	5 × 6 = 2	5 × 6 = 3	5 × 5 = 4
5½ × 5½	5 × 5 = 3	5 × 5 = 2	4½ × 5 = 4
5 × 5½ = 2	4½ × 5 = 2	4½ × 5 = 3	4½ × 4½ = 4 = 28
5 × 5 = 3 = 21	4½ × 4½ = 27	4½ × 4½ = 20	4 × 4 = 3
4½ × 5	4 × 4 = 4	4 × 4½ = 3	3½ × 4 = 3
4½ × 4½ = 2	3½ × 4½	4 × 4	3 × 3½ = 3
4 × 4 = 3	3½ × 4 = 3	3½ × 4 = 2	3 × 3 = 4
3½ × 4 = 2	3 × 3½ = 4	3½ × 3½	
3 × 3½ = 2	3 × 3 = 4	3 × 3 = 2	
3 × 3 = 2			

* Bag-like.

† The numbers in this column indicate the number of eggs corresponding to a given measurement.

TABLE 3

Showing detailed measurements on eight ovaries (autumn) tabulated in table 1. The line indicates the point of change in growth-rate

NO. 24	NO. 25	NO. 26	NO. 27
29 × 35	27 × 31	30 × 33	27 × 32
27 × 30	22 × 25	25 × 31	24 × 29
25 × 27 = 5	15 × 17 = 4	23 × 29	22 × 25 = 6
20 × 24	11 × 12½	21 × 24 = 7	18 × 21*
16 × 18		15 × 20	18 × 21
	6 × 7	11½ × 14	9 × 10½
6½ × 7½	6 × 6½	10½ × 12½	
6 × 7	5½ × 6½		5½ × 6½
+6 × 6½	5½ × 6 = 2	6 × 6½	5 × 6
6 × 6½	5¼ × 6 = 2	5½ × 6½	5 × 5½
5½ × 6	5¼ × 5½	4½ × 5½	5 × 5¼ = 8
5 × 5½ = 2 = 17	5 × 5½ = 20	4½ × 5½	4½ × 5
4½ × 5 = 2†	4½ × 5½	4½ × 5 = 2 = 16	3¼ × 3¼
4 × 5 = 2	4½ × 5	4 × 4½ = 2	3 × 3 = 2
3¾ × 5	4½ × 4¾	3½ × 4 = 2	
4 × 4 = 2	4 × 4½	3½ × 3½ = 2	
3¾ × 4	3½ × 4 = 3	3 × 3½ = 2	
	3 × 3¾ = 3		
	3 × 3½		

NO. 28	NO. 29	NO. 30	NO. 31
28 × 32	30 × 33	30 × 33	29 × 33
27 × 29	22 × 24	19 × 22	24 × 28
22 × 27	17 × 19 = 5	16 × 18 = 5	16 × 19 = 4
20 × 22 = 7	12 × 13	11 × 13	7½ × 8
15 × 18	7½ × 7½	7 × 8½	
12 × 13½			5½ × 5½
8½ × 9½	5½ × 5¾	6 × 6½	5 × 5½
	5 × 5¾	5 × 5	4¾ × 5
5½ × 6½	5 × 5½	3½ × 4	4½ × 5 = 2
5 × 6	4½ × 5 = 15	3 × 4‡ = 9	4½ × 4¾ = 21
4 × 5½	4½ × 4½	3 × 3¾	4 × 4 = 3
4½ × 5 = 14	4 × 4½	3½ × 3½ = 2	3½ × 4 = 3
4 × 4½	4 × 4 = 3	3 × 3¼ = 2	3¼ × 3½ = 3
3½ × 3½ = 3	3¾ × 4		3¼ × 3¼
3 × 3½ = 2	3½ × 3½ = 3		3 × 3¼ = 3
3 × 3 = 4	3¼ × 3½		3 × 3 = 2

* Not absolutely certain this egg belonged in this ovary.

† The numbers in this column indicate the number of eggs corresponding to a given measurement.

‡ Degenerating.

is water-poor, and rich in fat. At this same time moreover many new follicular cells must be formed. Within as short a time as five days a 6 mm. ovum may have grown to 33 mm. The relative sizes of the membranes for ova of the two stages are as 1 to 30. If all of the follicular cells arise from follicular cells—as seems probable—each cell must divide every twelve hours (10 divisions in 120 hours). It is certain that young and growing cells are abundant in the follicular membrane during the period of the rapid growth of the ovum. The chemistry of this membrane in the various phases of its activity, and the chemistry of the two forms of yolk which correspond to the two rates of growth here described, are the subjects of investigations to be published immediately in this series of papers.

It remains to point out that a strictly comparable change in growth-rate occurs during the "growth period" of other avian ova—very clearly in the pigeons—and indeed in the ova of many animals. The *time* of the change is most variable for the ova of different classes and phyla; but the extent of the change is probably nowhere as pronounced as in the birds.

His (8, p. 20) distinguished by their macroscopic appearance four classes of ova (Follikel) in the hen's ovary. The present data, and the data of an earlier paper (7) show that there are only the two above-mentioned natural groups when these are considered from a dynamic standpoint. It was further noted by His (p. 5) that the "number of follicles, even in hens that laid every day, is strictly limited." He tabulated the contents of three ovaries, finding in them 5–3–5 eggs respectively of more than 9.0 mm. in diameter.

D'Hollander (4) and Sonnenbrodt (5) have carefully studied the development of the ovum in the fowl and divided its growth into more or less distinct periods; but chiefly on the basis of nuclear phenomena. Sonnenbrodt (p. 418) describes also the appearance of the hen's ovarial egg and notes that they are "whitish till of about 5 mm. diameter . . . . the smaller ones rather grayish . . . . ; over 5 mm. were very yellow." This observer further found "up to six follicles over 10 mm. diameter, but mostly three or four."

Bartelmez (6) describes four stages in the development of the ovarial egg (pigeon). His classification is based upon the phases of activity of the oöcyte as a whole, and from any point of view in which size and changes in growth are being considered, his treatment is certainly the best the subject has received.

Sonnenbrodt and Bartelmez report a slow growth of the oöcytes to about 0.09 mm. where they (the earliest, at least) halt. The next period, according to Bartelmez carries the egg to a diameter of 0.4 mm.; but between this and 5.0 mm. no change in rate of growth is suggested. From 5 mm. to ovulation he finds the rapid growth which I had previously shown to begin at about 6 mm. in the fowl.

Agassiz and Clark (9) long ago pointed out that there is a final growth period in the eggs of turtles which is more rapid than that which preceded it; this more

rapid growth, lasting four years in all eggs; the first set of eggs beginning this rate in animals seven years old. In Lacerta, Braun and later Hoffman (10) showed that the formation of groups of ovarian follicles, with increased rate of growth, occurs only in the spring.

Many forms are known in which it has been practically certain that the final growth period is accomplished at an accelerated rate. A means of measuring this acceleration has however not hitherto been found. Moreover, the point at which it occurs has not been defined. Indeed, the term "final growth period" seems to have been rather generally applied to all the macroscopically visible ova of the ovary.

CONCLUSIONS

By suitable methods it has been possible to show that when the oöcyte of the fowl reaches a diameter of about 6 mm. it increases its previous rate of growth to a rate nearly 25.8 times higher.

The transition from the one rate to the other is made in a single day.

The transition sharply marks off the type of substance accumulated in the ovum. Under the slower rate this is white yolk; under the greatly increased rate the yellow yolk is produced.

The increased rate of deposition of yolk materials is necessarily accompanied by a pronounced alteration in the growth and activity of the membrane (follicular) which surrounds the ovum.

A comparable change in growth-rate occurs generally in other avian and sauropsidan eggs.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

II. On the Chemical Composition of White and Yellow Egg Yolk of the Fowl and Pigeon

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SOURCES OF INTEREST IN THESE DATA

The eggs of birds are known to contain yolk of two kinds. Because of their color the two kinds are called white and yellow yolk. The histological differences between the two forms are very marked and have long been known. The chemical differences are, however, known or indicated by only[1] a single partial analysis of white yolk reported by one of us (R. 1). The reason for the lack of analyses of the white yolk is doubtless to be found in the fact that the preparation of such samples is a most tedious process. Indeed, unless advantage is taken of the fact that the ova of the fowl consist wholly of white yolk until they begin their final period of rapid growth—described in Number I of these studies (2)—it is practically impossible to obtain such samples. When it is considered that the egg-nucleus, the germ, and the embryo are in direct contact with white yolk exclusively it becomes clear that we can know the chemical nature of the environment of the germs of birds only when the chemical composition of the white yolk is known. The analyses here reported demonstrate that the two forms of yolk are strikingly different substances, and that the white yolk much the more nearly approximates the composition of small holoblastic eggs, and of living undifferentiated tissue generally.

[1] The impression (e.g., Hammersten, Physiological Chemistry) that Liebermann (Pfluger's Arch. vol. 43) made even a qualitative analysis of white yolk seems to be an error. The blastodisc (Keimscheibe) which he thus investigated is a quite different structure and material.

An examination of the available data on the percentage composition of the yellow yolk indicates that the moisture, neutral fat and protein figures warrant new determinations. And, if a comparison is to be made with the figures obtained for the white yolk it is particularly desirable that the analyses of the two substances be carried out by identical methods. The phosphorus compounds of the egg have been much studied, but our analyses include determinations of alcohol-soluble water-insoluble phosphorus, as well as figures for the total extractive bodies, and ash. In nearly all previous analyses it seems practically certain that a recently established fact of importance has been much disregarded; namely, the fact that water is continually passing from the albumen to the yolk from the time the ovum leaves the ovary till the time of preparation of the sample. Greenlee (3) has shown that this exchange occurs from the time an egg is laid until many months thereafter, and that the rate of such absorption of water by the yolk is much greater in the very fresh egg than in the stale one. Our own data show furthermore that in a "fresh egg"—that is, in any "laid" egg—the yolk has already been very appreciably diluted by water from the albumen. The ovarian yolk contains less moisture than the yolk of the fresh laid egg. In few of the previous analyses is the exact age of the egg considered. This means that the data from those analyses represent the percentage of water as being both higher and more variable than is true for samples of pure undiluted yolk.

Little is known of the variation in chemical composition of the yolk produced by different orders, species and races of birds. This is true though there are some available data on the yolk of various *breeds* of the common fowl, and though Langworthy (4) has made four useful analyses of yolks from other species of poultry. Before convincing evidence on this point can be had it seems necessary that eggs not only fresh but of *identical* age (in hours), be taken for comparison; that eggs from more than one individual of a breed be used; and finally that the age, and previous egg-laying history of each bird be known and considered. These last-named points are justified by the data from about nine hundred analyses of individual yolks from eggs of various species of pigeons and doves, as this has been carried out by one of us (Riddle, unpublished data). A comparison of the chemical composition of the egg yolk of birds belonging to two different sub-orders of birds is afforded by the present data on the fowl and the pigeon—the analyses for both kinds of eggs having been carried out by a uniform method.

A further reason for interest in a comparison of the composition of

the two forms of yolk lies in the fact that the two forms have been shown (1 and 2) to be associated with two strikingly different growth rates; the two substances arise in the same cell under two quite unlike metabolic conditions. Our analyses show that the white yolk—which is stored but slowly in the cell—has a percentage composition approximately that of living growing tissues; and that the yellow yolk which is stored in the cell at a rate that is 25.8 times greater (see Number I of these studies) departs very widely in composition from all other substances or tissues.

METHODS OF PREPARATION AND ANALYSIS

The samples of yellow yolk from the laid eggs of common fowls and Jungle fowls were prepared as follows: The shell was divided along its longer diameter with a pair of small scissors. The two halves were used as 'boats' in which, alternately at first, the yolk was floated until freed from the greater part of the albumen. When a part of the albumen was thus removed the two chalazae were carefully cut away from their point of union with the vitelline membrane. The complete or final removal of albumen was accomplished by the use of elongated strips of hard filter paper which were repeatedly rolled under the yolk while still in its boat. In this way it is believed that all traces of albumen were removed from the yolk and from that half of the shell from which the yolk was finally released into the large weighing tube. The ovarian eggs were taken from birds as soon as these were dead. The outer theca was stripped from the ripest ovum; two samples (Y, Z) were then obtained by slitting the follicle at the hilum and forcing the last traces of yolk from the surrounding follicle. The third of these samples (M) was analyzed together with its inner follicular membrane as noted elsewhere.

The yellow yolk of the pigeon's eggs was obtained by steaming the just-laid eggs for eight minutes, cooling in water for four minutes, and then completely removing the albumen and vitelline membrane. These samples are quite clean, and comparisons with the uncoagulated (pigeon) yolks rolled on filter paper show that even the moisture values of the yolk are unmodified.

For the preparation of the white yolk samples several entire ovaries were obtained from laying hens; the ovaries were removed as soon as the birds were dead. All of the normal ova having a diameter of 3 to 6.5 mm. were separated from the ovary, cleaned, their outer theca

removed, slit open, and their contents pressed out and combined in in a weighing tube. The operation is a tedious one.

The methods used in the analysis are those described in detail by Koch (5). In brief, the material was extracted four hours with alcohol, one hour with ether (then very finely pulverized), and finally twelve hours with alcohol, in a Koch extractor. Water-soluble organic and inorganic bodies were separated from both fractions resulting from the alcohol-ether extraction. The phosphorus ofthe alcohol-ether extract (exclusive of the water soluble-portion)[2] was determined by the Neumann-Pemberton method, and calculated as lecithin (phosphatides).

The figures given for neutral fat and protein were obtained by difference.[3]

CONSIDERATION OF PREVIOUS RESULTS

A single analysis by Parke (7) supplies the figures which are quoted in all text books as representing the percentage composition of egg yolk. Three yolks were combined in the sample. The moisture content as given is certainly too high, probably for the reason already noted. The extraction methods of Parke's time probably did not effect a complete separation of the lipoids. Only 68.6 per cent of the solids were alcohol-ether soluble according to Parke. In our analyses this fraction is always closer to 70 per cent.

More recently rather extensive analytical data on egg-yolk—particularly on the protein of egg yolk—have been obtained by investigators at the Connecticut State Experiment Station, and at the United States Government Laboratories. The quantity and distribution of various nitrogenous bodies have been studied in detail. Percentages of the grosser composition have however received less attention. Langworthy gives in connection with his results moisture values which are high. The yolks analyzed were from eggs obtained in the market.[4] The value of the alcohol-ether soluble fraction is slightly higher than

[2] This fraction from egg-yolk contains only a trace of phosphorus. In five of the analyses of the pigeon's yolk the phosphorus was determined for the entire alcohol-ether soluble fraction.

[3] Atwater and Bryant (6), state (p. 53) that "it is probable that the factor 6.25 is not correct for eggs" and that "the value of protein by difference is perhaps the more nearly correct."

[4] The object of the investigation was to learn the composition of eggs as these are obtained for food purposes.

was obtained by Parke. This author also analyzed the egg yolk of the duck, goose, turkey and guinea-fowl. Since these yolks too were from eggs obtained in the market, and hence of unknown age, the moisture values are doubtless too high. Langworthy's results upon the yolks of these species, recalculated into per cent of solids, are as follows:

BIRD	WATER	FAT	PROTEIN	ASH	CARBOHYDRATES*
Duck	(45.8)	66.8	31.0	2.0	0.3
Goose	(44.1)	64.6	31.0	2.3	1.3
Turkey	(48.3)	63.6	33.6	2.3	0.8
Guinea fowl	(49.7)	63.2	33.2	2.4	0.8

* The percentages of carbohydrate are apparently based on the moist weight. The amount given for the hen's egg is 0.67 per cent.

It is stated that individual samples vary more or less and that the above figures are averages. There would seem to be real differences in the composition of yolk, for instance, from the duck and guinea fowl. It seems to us probable that if the moisture values had been obtained from ovarian, or just-laid eggs that the moisture differences would here have been—as we found in the case of the fowl and the pigeon—the most marked of all the differences. Cook (8) obtained a moisture value of 45.3 per cent from a sample containing a number of yolks, and a second value from a similar sample of 48.5 per cent. He also gives data for the ash, and for protein as calculated from total nitrogen. Pennington (9) made very extensive studies, particularly on the distribution of the nitrogen of the yolk. The moisture values given are higher than we find for the undiluted yolk; and the extract obtained with alcohol and petroleum ether is somewhat smaller than the amount we obtain.

Malcolm (10) has published some data on the percentage composition of egg yolk. For our purposes, however, the results are hardly usable for three reasons. (1) Because it seems that entire yolks were not used; and with this method of pipetting out portions of the yolk it is quite possible to include, or to fail to include, the white yolk at the center of the egg, and this will certainly introduce considerable variations—as was found by Malcolm—in the percentage of lecithin obtained. (2) The age of the eggs used is unknown. (3) The method of correcting the yolk-weight by pro-rating between yolk and albumen the loss of water from the egg before beginning analysis is wholly wrong; it magnifies instead of minimizes the error in the determination

of the true initial weight of the yolk. As already noted it has been found that the yolk is constantly absorbing water from the albumen instead of losing water to the albumen as was assumed by Malcolm.

Serono and Pallozzi (11) made partial yolk analyses of fowl's eggs less than twenty-four hours old, after removing the albumen from the yolks by rolling the latter on filter paper. They did not make analyses of individual eggs but combined ten yolks in a sample. Their fat extrac-

TABLE 1

Analyses of yolk of fowl's egg by various investigators

AUTHOR	REMARKS	MOISTURE	LECITHIN	FAT	PROTEIN	ASH
Parke	Per cent of moist weight	47.20	10.72*	22.82	15.63	0.97
Langworthy	Per cent of moist weight,	49.5	33.3		15.7†	1.1
Malcolm	Per cent of moist weight		27.79 to 31.89		17.50‡	
Serono and Pallozzi	Per cent of moist weight		11.05 to 12.09	18.87 to 21.47		
Cook	Per cent of solids	45.39§ and 48.74§			30.10† and 31.87	2.11 and 2.34
Pennington	Per cent of moist weight	47.43 to 48.03¶	31.44 to 33.33			1.31 to 2.58
Riddle	Per cent of solids	47.8	20.9*	49.2	28.8	
Greenlee		47.35				

* Phosphorus of alcohol-ether extract calculated as lecithin.

† These figures = N × 6.25.

‡ This figure = the average N of 18 samples, × 6.25 (18.22, the calculated average weight of the yolks of 18 eggs).

§ The samples each contained more than one—an unnamed number—of eggs.

¶ Six to eighteen eggs used in a sample; a total of 19 analyses.

tion seems to us inadequate, and the amount of fat obtained by them is less than that obtained by us. The amount of fat which they obtained—in spite of the large combined samples used—was also so variable as to raise much doubt as to the adequacy or accuracy of the extraction methods used. They give the minimum and maximum of fat (+lutein) obtained as (nearly) 18.87 and 21.47 per cent of the moist weight of the sample. Their figures for lecithin are, on the average (11.57 per

cent moist weight), slightly higher than those obtained by us. We fail to obtain moisture and protein values from their results, which were concerned chiefly with the lutein fraction of the yolk. These workers used especially elaborate precautions against the occurrence of changes in the lipoids during the analysis.

To facilitate a comparison of the results obtained by these several investigators table 1 has been prepared. The data of the table also, in some respects, amplify the foregoing brief statement. These tabulated data are moreover in convenient form for comparison with our own results. Studies similar to ours on the composition of the yolk of the pigeon's egg seem not to have been made hitherto.

DISCUSSION OF THE DATA

Yellow yolk[5] *of the common fowl.* The results of six analyses are given in table 2. An examination of the several moisture determinations shows that egg yolks obtained from the ovary and oviduct contain the smallest amounts of water. The amount is probably 2 per cent less than the figures obtained by one of us (1) and by others on "fresh" eggs. Greenlee showed that in laid eggs, kept at ordinary temperatures, solids do not pass from the yolk to the albumen. It is clear therefore that when the yolk leaves the ovary and becomes surrounded by albumen it then becomes contaminated with water absorbed from the albumen. Uncontaminated natural yolk contains little more than 45 per cent of moisture. If all of the alcohol-soluble water-insoluble phosphorus exists in the form of lecithin this substance forms 21.9 per cent of egg yolk, and the neutral fat is equal to 47.3 per cent. The amount of protein in egg yolk is 29.1 per cent.

[5] Samples of really pure yellow yolk cannot be obtained from any bird; a single full-grown ovum,—an egg yolk entire—forms each sample designated yellow yolk in table 2. At the center of each yolk there is a core—about six mm. in diameter—of white yolk; this is equal to about 1/160 of the total volume of a yolk of 33 mm. diameter. Extending from this central core to the germinal spot (or vesicle) at one point of the yolk's surface is also a cylinder of white yolk; this includes perhaps less than 1/200 of the entire volume of the egg yolk. There are furthermore five to ten quite thin strata or layers of white yolk between the central white yolk core and the periphery of the ovum; it is uncertain what proportion of the total volume of the egg is included within these strata, but probably it is as much as 3 or 4 per cent. It is clear therefore that the "yellow yolk" of our analyses is probably contaminated by about 5 per cent of white yolk The differences between the two forms of yolk are therefore somewhat greater than our analyses indicate. The eggs of White Wyandotte and Barred Plymouth Rocks were used.

TABLE 2

Percentage composition of yolk of fowl and pigeon

NO. OF ANALYSIS	REMARKS	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS					H_2O
			Phosphatides	Neutral fat	Protein	Organic extractives	Ash	
Yellow yolk of common fowl								
M	From ovary*	10.075	22.25	49.50	26.50	0.91	0.84	46.89*
M	M corrected	9.716	22.32	49.78	26.54			45.82
Y	First of second ovary	15.405	69.41†		30.59†	←	←	45.43
Z	Second of third ovary	8.520	18.44	49.83†	31.73†	←	←	47.17
A	From oviduct	16.20	21.90	47.26	29.10	0.56	1.19	45.38
N	From oviduct	12.822	19.15	51.88	27.23	0.86	0.83	46.99
D	Laid egg + 48 hours.	17.67	20.70	49.40	28.77	0.62	0.53	47.80
White yolk of common fowl								
R	From ova, 4.5 to 6.5 mm.	6.018?‡	12.66	42.25	35.15	3.88	6.03	89.65?
48	From (93) ova 3 to 6 mm.	2.688?‡	11.00	23.11	44.44	8.60	12.88	88.36?
Yellow yolk of jungle fowl								
20	Laid + 48 hours	10.880	67.30		29.09	2.03	1.60	48.73
23	Laid + few hours	10.883	19.90	46.74	30.47	1.59	1.30	48.70
Yellow yolk of common pigeon								
760	First + 4½ hours	3.885	20.54	69.80	30.20†	←	←	55.16
761	Second + 2 hours	3.930	20.75	68.69	31.31			54.75
886	First + 5 hours	3.272	21.67	67.65	32.35			57.17
887	Second + 5 hours	3.502	19.37	67.30	32.70			56.50
110¶	Second + 3 hours	2.813	20.15	67.55	32.45	2.00	2.11	54.21

* This, the largest yolk in the ovary of a laying hen, was covered with its inner follicular membrane. Twelve similar membranes weighed 3.110 grams and had a moisture value of about 88 per cent. The moisture figure given for this sample is therefore slightly too high for the yolk alone. Corrections made necessary by the presence of this membrane are applied in the next column.

† This figure includes ash and organic extractives.

‡ On account of the very considerable time consumed in preparing the white yolk sample it is not possible to be sure that the original moist weight is correct.

¶ Egg of wild *C. livia*.

TABLE 3

Moisture determinations (by drying at 100°C. and cooling over H_2SO_4) of series of ovarian eggs

	LAID EGG		FIRST OF OVARY		SECOND OF OVARY		THIRD OF OVARY		FOURTH OF OVARY		FIFTH OF OVARY	
	Weight Yolk	Per cent H_2O	Weight	H_2O	Weight	H_2O	Weight	H_2O	Weight	H_2O	Weight	H_2O
Series 1..	12.335	47.97	12.295	lost	8.520*	47.17	4.290	47.72				
Series 2..	14.9747	47.21	15.405†	45.43	12.499	45.40	8.684	45.29	5.076	45.72	1.514	48.48

* This = analysis "Z" of table 2.
† This = analysis "Y" of table 2.

Before considering the remaining sections of table 2 the moisture determinations on two series of ovarian eggs may be noted in table 3. It will be observed that those data confirm the statements made above on the smaller water content of ovarian eggs. They reflect an additional point, namely, that ova of 4 grams and less show an increase in moisture due to the relatively high proportion of white yolk in these eggs. In the following section it will become clear that this white yolk has a very high moisture value—equal to that of egg-albumen.

White yolk of the common fowl. The two analyses of white yolk give figures (table 2) which strongly contrast with the above figures for yellow yolk. One of the analyses (R) presents a less striking contrast than does the other (48). We believe this to be due, in part at least, to the fact that some ova of 6.5 mm. diameter were used in the preparation of this sample, and that such ova really contained some yellow yolk. It will be noted that *all* of the fractions from this analysis are intermediate in amount to the corresponding fraction of yellow yolk on the one hand, and to those of the less questionable sample of white yolk on the other.

It will be noted that the composition of white yolk is by no means so far removed from that of embryonic tissues as is that of the yellow yolk. The white contains but half of the neutral fats and phosphatides found in the yellow. Protein, extractives, and ash are much more abundant in the white yolk. The ratio of water to solids in the two forms shows an even greater contrast; for the yellow this ratio is, 45 : 55; for the white it is, 89 : 11.

Yellow yolk of the Jungle fowl. The Jungle fowl is usually considered the ancestor, or one of the ancestors, of the many breeds of domestic fowl. Our two analyses of yolks from Jungle fowl eggs show that in

these yolks the moisture value is wholly similar to that of "fresh" eggs from various breeds of the common fowl. The amount of alcohol-soluble materials would seem however to be perceptibly less; the amount of protein appreciably greater.

Yellow yolk of the common pigeon. The five analyses here reported demonstrate a striking difference in the moisture values of the egg yolk of the fowl and the pigeon. This amount is nearly 10 per cent

TABLE 4

Averages, or most reliable percentages, of forms of egg yolk

NATURE OF YOLK	CALCULATIONS IN	WATER	PHOSPHATIDS	NEUTRAL FAT	PROTEIN	ORIGINAL EXTRACTIVES	ASH
Undiluted yolk of common fowl (yellow)	Per cent of solids*		20.58	49.51	28.36	(0.70)	(0.85)
	Per cent moist weight	45.40	11.15	25.25	15.04	(0.36)	(0.44)
Undiluted yolk of common fowl (white)	Per cent of solids		10.91	23.11	44.44	(3.88)	(6.03)
	Per cent moist weight	86.66	1.13	2.39	4.60	(0.40)	(0.62)
"Fresh"† yolk of Jungle fowl (yellow)	Per cent of solids		19.90	46.70	29.78	2.03	1.60
	Per cent moist weight	48.71	10.21	23.96	15.27	1.04	0.82
"Fresh"† yolk of common pigeon (yellow)	Per cent of solids		20.50	47.00‡	29.00‡	2.00	2.11
	Per cent moist weight	55.56	9.11	20.81‡	12.89‡	0.89	0.94

* Only in the figure for water are the "fresh-laid" eggs excluded from the averages.

† I.e., yolk taken from an egg within a few hours after the egg was laid.

‡ These figures known to be approximately correct partly from data not included in table 2.

greater in the latter. The yolk of the common pigeon contains, moreover, a smaller percentage of water than has been found in most other species of pigeons and doves. It is therefore certain that two quite different moisture values characterize the eggs of the *Gallinae* and the *Columbinae*.[6]

[6] Langworthy's data for representative species of *Gallinae* support this conclusion.

The data warrant the further conclusion that the egg yolk of the pigeon contains less alcohol-soluble material, and more protein, than does the yolk of the common fowl. These figures for the pigeon more nearly approach those obtained for the Jungle fowl.

Averages and most reliable determinations. We believe that the most accurate account of the percentage composition of the various forms of yolk—as analyzed by us—cannot in all cases be obtained from the average of the several determinations. The variation of moisture due to the length of time the yolk has been in contact with albumen, and the probable contamination with traces of yellow yolk of one of the white yolk samples, are examples of probable inaccuracies that may be introduced by the 'average.'

In order to present what we believe to be the most reliable figures obtained for the various forms of yolk table 4 has been constructed. We there give also the percentages calculated in terms of moist weights in order to facilitate a comparison of our results with those of others who have presented figures based upon moist weight only. The summary form of this table will further assist the reader in making a general comparison of the gross chemical composition of the two kinds of yolk, and of yellow yolk from the three different sources.

CONCLUSIONS

The moisture values found when "fresh" laid eggs are taken for analysis are always too high. The yolk of the bird's egg begins to take up water from the albumen as soon as it leaves the ovary and enters the oviduct.

The ovarian egg (yellow yolk) of the fowl contains little more than 45 per cent of water. The solids of the fowl's egg contains 20.6 per cent of phosphatides, 49.5 per cent neutral fat, and 28.4 per cent protein.

The white yolk markedly differs from the yellow yolk in all of the six fractions studied. It is much less like "yolk" and much more like embryonic tissue in its composition. The egg-nucleus, the blastoderm and the embryo are, at all stages, in immediate contact with the white yolk—not with the yellow yolk. It is clear therefore that the medium with which these living elements are immersed is not radically different in gross composition from the living elements themselves.

The egg yolk (yellow yolk) of the Jungle fowl has probably a lower lipoid, and a higher protein content, than the yolk of domestic fowls.

The water content is probably equivalent in the (supposed) ancestral and derived forms.

The egg yolk (yellow yolk) of the pigeon differs most from that of the fowl in its much higher moisture value. Probably differences in the amount of alcohol-soluble and protein materials also exist.

The yellow yolk derived from different orders, genera and species of birds probably varies more in the amount of water than in other fractions. But the yellow yolk from birds of different orders is relatively much alike as compared with the yellow and white yolk from the same individual bird.

The extreme divergence in composition of these two forms of yolk—as deposited in the *same cell* of the individual bird—is definitely associated with two strikingly different grades of metabolism within the cell. The yellow yolk, with its very low water value, is produced when the storage powers of the cell are extremely high; the white yolk—with a water value higher than, or equivalent to, that of milk—is produced when the storage processes of the cell are much reduced.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

III. On the Metabolism of the Egg Yolk of the Fowl During Incubation

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In Liebermann's (1) very extensive study of the metabolism of the fowl's egg the entire extra-embryonic contents of the egg were combined and analyzed together. No attempt was made to follow the changes in the yolk apart from the albumen and the fluids of the amnion and the allantois. Later work has not supplied such data. The yolk and the albumen are wholly unlike substances, produced in different organs; the allantois presents during development increasing accumulations of the non-gaseous end-products of metabolism; and the amniotic fluid arises at the expense of other of the egg contents. It is therefore clear that each of the groups of substances runs a separate course in the developing eggs and that the picture obtained from a study of all of them combined may very imperfectly reflect the course of events in any one of the group. The present study deals with changes in the *yolk* during incubation. Our chief problem was to determine the relative rates at which the various constituents of yolk are removed and utilized by the embryo.

Such a study requires the isolation and chemical analysis of the yolks of eggs subjected to various periods of incubation. The clean and perfect isolation of yolk samples from the incubating egg offers a number of difficulties, some of which it is necessary to describe. (1) At all stages the presence of a certain amount of living tissue—the yolk-sacs—offers a complication. It would be difficult to obtain all adhering traces of yolk from the yolk-sac, and since considerable quantities of yolk are met with in large intracellular spaces (intra-

cellular yolk) of the sac it seems highly desirable to analyze the yolk-sacs along with the unabsorbed yolk of the various stages of incubation. But this combination of living tissue with unmetabolized yolk-residues requires additional and separate analyses of the yolk-sac tissues of the various stages being studied, in order that one may know how much and in what direction the presence of this tissue affects the figures obtained for the yolk analyses. (2) The yolk-sac completely encloses the yolk on the twelfth day of incubation. Previous to this stage the vitelline membrane at the lower pole of the egg is so fragile as to make the separation from albumen an uncertain and difficult task. We have therefore confined our study to periods beginning with the twelfth day. (3) Even after completely covering the yolk the yolk-sac continues to grow. During this same period the amount of yolk rapidly diminishes. The relative proportions of these two materials therefore steadily changes during incubation. (4) More or less solid and viscous *bodies* were found in a fraction of the yolk-sacs of very late stages of incubation. Such bodies are surrounded by a much more fluid yolk. The presence of one or more of these bodies in an analysis of yolk-sacs plus their undigested residues will very considerably influence the results; for, two such bodies separately analyzed (41 and 35 of table) differ quite markedly from all other forms of yolk at a corresponding stage of incubation. We are not sure that such bodies were absent from all of the analyses of yolk-residues of intact yolk-sacs.

The methods of analysis were those of the preceding paper of this series of studies (2). A full description of the methods has been given by Koch (3). The phosphorus of all of the analyses was calculated as lecithin (phosphatides).

THE YOLK-SAC AND ITS CHEMICAL COMPOSITION

The yolk-sac of the chick is a highly vascular, glandular, entodermal (splanchnopleure) membrane which by the twelfth day of incubation has completely surrounded the yolk; it has also separated with approximate completeness the yolk from the remnant of egg albumen. The twelve day stage was for this reason selected as the first stage for chemical study. Fully formed membranes and clean samples of the contained yolks may both be obtained at this period. The yolk-sac with its residue of yolk is stated by Virchow (4) and others to be drawn within the body of the chick usually on the nineteenth day of incu-

bation, but to reach its highest development a little later,[1] and to maintain its highest development to at least a day or two after hatching. Yolk-sacs of the nineteen day stage were therefore selected as the second or later stage for study. In collecting the samples, however, it was observed that the yolk-sacs sometimes remained outside of live embryos of even twenty-one days of incubation; a few samples of twenty and twenty-one day stages were therefore also collected[2] and analyzed.

From the preceding statement it is evident that there is no reason for identifying any one of these stages of the yolk-sac with greater or less *activity* than another stage.[3] In accord with the inference that the two stages are functional equivalents, the analytical data from the samples of twelve and nineteen day membranes (45 and 32 of composite table) are rather closely similar. The twenty-one day preparation (F) was much smaller than the preceding, and unlike those samples was not freed from "intra-cellular" yolk. For these reasons it is improbable that the apparent divergence in the composition of this stage from earlier stages is real. The differences are those to be expected from our failure completely to free this sample of yolk.

The three stages of this membrane were analyzed not primarily for comparison with each other, but for the purpose of a check upon the figures obtained for the analyses of the yolk-sacs plus their unas-

[1] A comparison of the weights of twelve day and nineteen day yolk-sacs whose combined weights are given in the table (45, 32) plainly shows that the latter are much larger. The increase in size of the yolk-sac after the time of complete enclosure of the yolk (twelve days) is chiefly due to the extension of the folds or septa of the inner wall. The dimensions of the external boundary of the yolk-sac are probably little changed during this period.

[2] Large numbers of eggs of white Wyandottes were incubated to appropriate stages. Where volk-sacs free from their volk contents were wanted the sacs were freed from albumen and umbilical connections and rinsed rapidly in physiological salt solution followed by dipping in distilled water and then removing the surplus moisture with filter paper. The sacs were then slit entirely open and the interior flushed and cleaned in a similar way. There remained, particularly in the eggs incubated beyond the twelve day stage, evident collections of "intracellular yolk" within the walls of the yolk-sac. These points were punctured and all such visible yolk was pressed out and flushed with the cleansing fluids. Where the yolk-sacs plus the contents were used only the external cleansing was required. For the several samples of fluids or bodies taken from within yolk-sacs no special treatment whatever was necessary.

[3] The yolk-sac is actively functioning and actively growing in both the twelve and nineteen day stages.

similated residues of yolk.[4] The ratio of phosphatides to neutral fat in the yolk-sac tissue will be seen (first section of table) to be somewhat higher than in egg yolk; the ratio of the former being about 7 : 9; of the latter 2 : 5. Nearly 75 per cent of the yolk-sac tissue is protein.

YOLK NOT METABOLIZED BY CHICK EMBRYOS OF 12, 18, 20, AND 21 DAYS

The results of analyses of yolk of these four stages are given in the second and third sections of the Composite Table. A yolk of twelve days incubation (26) shows that the proportions of lecithin, neutral fat and protein remain practically as they were in the fresh yolk. The proportion of water has been increased about 9 per cent. The amount of these substances in pure unmodified yolk was shown by Spohn and Riddle (2) to average as follows: Lecithin, 20.58 per cent; neutral fat, 49.51 per cent; protein, 28.36 per cent; water 45.40 per cent. The yolk of twelve days incubation yielded 20.07 per cent lecithin; 48.60 per cent of neutral fat; 27.56 per cent protein; and 54.76 per cent of water. There is little doubt that up to the twelfth day of incubation the various solids of the yolk are removed from it in not very unequal proportions.

Between the twelfth and eighteenth days, however, the lecithins and fats are mobilized to a greater extent than the proteins. These facts are plainly shown by analyses *G* and *H* (see table). Our data show further that during this period the proportion of water has suffered a reduction of nearly 9 per cent, i.e., to the normal proportion for unmodified yolk. In connection with these data for twelve day and 18 day incubations reference may be made to a single analysis by Parke (5) of a yolk incubated for ten days, and of another incubated for seventeen days. Unfortunately Parke fails to say anything whatever of the preparation of these samples, and his extraction certainly failed to separate all of the fat. His moisture determinations, however, are doubtless of value. In the ten day egg this was found to be

[4] Six such analyses are reported in the second section of the table. An examination of the weights of these samples will show that the amount of membrane present in each is almost negligible for some of the determinations. The data are so placed in the tabulation, however, that the direction in which the living tissue influences the figures for the entire sample need in no case be wholly overlooked. Estimates of the proportion of membrane present in each of these analyses are given in the form of a fraction placed in parentheses immediately underneath the moist weight of the several samples.

Composite table

	NATURE OF TISSUE	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS					H_2O
			Phosphatides P as lecithin	Neutral fat	Protein	Organic extractives	Ash	
45	Yolk-sacs (41) 12–14 days incubation	1.055?	7.47	8.23	74.82	4.06	5.42	74.56
32	Yolk-sacs (13) 19–20 days incubation	1.150+	7.17	10.25	74.52	3.64	4.42	70.40
F	Yolk-sacs (3) 21 days*	0.482	trace	29.09	65.06	3.33†	2.52†	74.48
26	Yolk-sac (J. F.) and contents‡ 12 days incubation	11.090 (1/120)§	20.07	48.60	27.56	2.02	1.75	54.76
G	Yolk-sacs (3) and contents 18 days incubation	34.096 (1/60)	16.81	45.67	37.86	0.45†	0.23†	45.68
H	Yolk-sacs (3) and contents 18 days incubation	35.275 (1/60)	13.25	38.78	46.73	0.69†	0.53†	46.48
35	Yolk-sac and contents 20 days incubation	13.775 (1/40)	10.82	26.73	59.61	1.61	1.23	52.09
36	Yolk-sac and contents 20 days incubation	15.448 (1/40)	14.74	38.19	44.86	1.08	1.13	47.75
E	Yolk-sacs (3) and contents 21 days incubation	9.450 (1/15)	17.62	47.26	32.73	1.39	1.16	56.52
37	More liquid contents from yolk-sac (20 days)	4.262	11.76	33.09	51.88	1.96	1.31	44.17
39	More liquid contents from yolk-sac (20+ days)	4.624	11.23	30.02	55.25	2.00	1.49	54.18
41	Central yolk body (less viscous) of 20 days yolk-sac	0.800	19.68	46.05	28.87	2.00	3.40	37.13
34	Central yolk-body (quite viscous) of 19 days yolk-sac	2.532	3.95	8.71	82.60	2.23	2.51	71.27
33	Intracellular yolk and yolk-sac at 19 days	2.280 (1/10)§	8.91	64.83	18.80	4.27	3.19	78.84

Composite table—Continued

	NATURE OF TISSUE	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS					H_2O
			Phosphatides P as lecithin	Neutral fat	Protein	Organic extractives	Ash	
O	Resorbed ovum (from hen).....	1.834	15.30	45.65	35.26	2.08	1.71	63.20
5½	"Extra foll. yolk" (fowl).	1.605	19.05	45.49	26.21	3.47	5.78	74.22

* Not really freed from fat; the "intracellular" yolk not pressed out.

† The figure is for the alcohol-soluble substance only.

‡ This and following five analyses were of yolk-sacs and their contents entire.

§ The fractions of this column are estimates of the amounts of living tissue (sac) present.

We are under obligations to Miss Adelaide A. Spohn for assistance with this series of analyses.

10 per cent higher than in "fresh yolk;" in the seventeen day egg the moisture was reduced nearly 13 per cent from the ten day level, or nearly 3 per cent below that of fresh yolk. In general, this result accords with our own data on the fluctuations in the moisture value during this period.

The data for the yolk residues attached to twenty day embryos show (35, 36) that the preponderant utilization of lecithin and fat is continued to this stage. The amount of these substances being proportionately still further reduced, while the relative amount of protein is still further increased. The amount of water in this and the twenty-one day stages again unmistakably increases. The relative amount of protein in the twenty day stage is nearly double that present in fresh yolk; the proportion of phosphatides[5] is slightly less than three-fifths that found at the beginning of development; the relative amount of neutral fat present is a little more than three-fifths of the amount originally present. In all (seven) of the analyses of eighteen

[5] Liebermann did not separate or determine the phosphorus or phosphatides in his analyses; his figures for protein apply, as previously noted, to all the extra-embryonic contents of the egg—not to the yolk alone; and the proportion of ether-soluble substance cannot be stated or followed, as we have done above, because the original weight of yolk-substance alone was undetermined, as was also the amount of extraneous protein which formed a part of his "total solids." We are therefore unable to use any of Liebermann's data in the comparisons made above.

to twenty-one day stages the phosphatides show a somewhat greater reduction than do the neutral fats.

Two analyses (37, 39) of yolk that could be poured from slitted yolk-sacs of twenty, and twenty and one-half days incubation fully confirm the above findings on the relative proportions of yolk subtsances at this stage. The phosphatides here average 11.5 per cent; the neutral fat 31.5 per cent; the protein 53.5 per cent. The moisture values here are doubtless somewhat too low since this yolk was directly exposed to the air while being collected from the sacs and weighed. The figures are 44.2 per cent and 54.2 per cent and here again the egg that had gone somewhat beyond the twenty day stage has the considerably higher moisture value.

The single analysis (E) of yolk of twenty-one days incubation shows only a part of the considerable reduction of phosphatides (17.6 per cent) noted in the immediately preceding stages. Moreover, the neutral fat (47.3 per cent) is only slightly less than that found in fresh yolks, and the amount of protein (32.7 per cent) only a little higher. Attention may, however, be called to the fact that the amount of yolk in the yolk-sac of this stage was quite small in comparison with the amount present in the eighteen and twenty day sacs; and further that it is unusual (abnormal?) to find the yolk-sac still external to the embryo—as these were—after the twentieth day of incubation.

Our data indicate that in the period during which the greater part of the yolk is mobilized—the twelfth to the twentieth day of incubation—the phosphatides are metabolized more rapidly than other substances. The neutral fats are utilized more rapidly than the proteins; this holds true for the eighteen to twenty day period, in which the protein of the yolk is the sole source of protein for the embryo.[6] The amount of moisture in the yolk increases to, or beyond, the twelve day stage; between the twelve and eighteen day stages it is decreased; it is probably again increased at or near the very end of yolk absorption.

UNUSUAL FORMS OF YOLK—AND YOLK METABOLIZED IN SITUATIONS OTHER THAN IN YOLK SACS

Reference has already been made to the occasional presence of semi-solid or viscous bodies in yolk-sacs at the close of incubation.

[6] The egg albumen which surrounds the yolk entirely disappears at about the sixteenth or seventeenth day of incubation.

to be wholly different in their composition. The smaller, less viscous Two such bodies were analyzed (see 41 and 34 in table) and proved body from a yolk-sac of twenty days incubation, was practically normal unchanged yolk; little changed except in its moisture value which was about 13 per cent too low for yolk of this stage of incubation, and the lowest thus far found in any form or stage of yolk.

The larger more viscous yolk-body was removed from a yolk-sac of nineteen days incubation. In its composition it bears much less resemblance to normal yolk than to the living tissue of the yolk-sac. This fact becomes quite plain by reference to the table. The moisture value of this body was extraordinarily high—71.3 per cent. This value exceeds by more than 15 per cent the moisture of any other form of yolk which we have obtained from *within* a yolk-sac.

The fact that in late stages of incubation we sometimes meet with bodies so unlike chemically as the two just described is certainly a fact of importance in any chemical study of the yolk-residues of this period. In the later samples collected by us the sacs were manipulated between the fingers to assure ourselves of the absence of these semi-solids. The origin of these bodies is quite unknown.[7]

Yolk which has passed through the innermost layer of cells of the yolk-sac epithelium is found in masses of considerable size at many points on yolk-sacs of late incubation stages. It is of interest to know the composition of such quasi-mobilized yolk. The single analysis (33) we have made of this substance indicates that it is a highly modified yolk. The phosphatides (8.9 per cent) are not quite half that of normal yolk. Curiously, however, the proportion of fat is considerably higher (64.8 per cent) than that of normal yolk. The relative amount of protein is only two thirds (18.8 per cent) that of fresh yolk and less than half the amount of protein of yolk-residues of this stage of incubation.

Yolk is sometimes formed in a situation quite outside of eggs as

[7] One may conjecture that those viscous bodies which have a composition approximating that of pure yolk were formed by a rather prolonged and complete *halting* of the growth of the egg when it had attained a diameter of perhaps 10 mm. or 12 mm.; and that normal yolk deposition was later resumed about this central modified core (most of the eggs used for this work were summer eggs, and the birds were laying by no means regularly). It is perhaps conceivable that the bodies of the type which differs so radically from yolk, are pinched-off septa of the inner wall of the yolk-sac which are later infiltrated with traces of yolk; or, that they represent the final clump of egg-albumen driven into the yolk and partly infiltrated with it.

was shown by me in an earlier publication (6). Yolk thus formed, and collected from free spaces of capsules which have liberated ova, is shown by a single analysis (57) given in the table, to be true yolk. The moisture value of this yolk is however extremely high, namely 74.2 per cent. The slightly lower percentage of phosphatides, fat and protein is probably accounted for by the abnormally high ash and extractive figures. These latter figures are probably too high because of the smallness of the sample.

In those cases in which nearly full grown ova are resorbed by the follicle which surrounds them, it is possible to study in a totally different situation the relative rates at which the constituents of yolk are mobilized. Only one analysis of such yolk has been made. This furnishes, however, a very interesting parallel to the results obtained from the above described studies on yolk of the yolk-sac. Here again (see analysis *O* of table) the phosphatides were removed most rapidly; the neutral fats next most rapidly, and the proteins slowest of all. The moisture value (63.2 per cent) here also is much increased.

CONCLUSIONS

The composition of the yolk-sac of the fowl was determined for the twelve and nineteen day stages of incubation.

A differential utilization of the elements of yolk prior to the twelfth day of incubation has not been shown to occur.

A study of the unmetabolized yolk of twelve, eighteen and twenty day stages shows that after the twelfth day the phosphatides are utilized more rapidly than the neutral fats. And the neutral fats are utilized faster than the proteins. This order of utilization of these substances persists during the eighteen to twenty day period when the embryo's sole source of protein is the protein of the yolk.

The moisture value of the yolk undergoes very considerable fluctuations during incubation. At the twelfth day it is about 9 per cent higher than in fresh undiluted yolk. At the eighteenth day it has fallen to near the value for fresh yolk. At the very end of incubation this value probably rises considerably.

Two forms of semi-solid yolk bodies which are occasionally present in final stages of incubation were found to be wholly unlike in their chemical composition.

Yolk resorbed by the follicle which secreted it shows—like yolk mobilized from the yolk-sac—a more rapid utilization of the phospha-

tides (lecithin); the neutral fats are utilized at a somewhat slower rate; while the proteins are metabolized more slowly than either the phosphatides or neutral fats.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

IV. When a Gland Functions for the First Time is its Secretion the Equivalent of Subsequent Secretions?

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The question of differences in composition of the initial secretion of a gland, as compared with subsequent secretions from the same gland, seems hitherto not to have been investigated. The nearest approach to information of this kind concerns the product of the mammary glands. It is obvious, however, that it is not possible to study the very first secretion of these glands since they function to a greater or less extent at or near the time of birth, and this earliest secretion is too small in amount, as well as too involved in time relations, to permit a quantitative analysis. Moreover few if any of the glands of the mammalian body can be utilized for such a study.

In the albumen-secreting gland of the bird's oviduct, however, it is easily possible to know when the gland *first* secretes albumen, since this coincides with the time of liberation of the first ovum from the ovary and the time of laying of the first egg. Too, the amount of the secretion is here sufficient for chemical analysis. The egg-albumen of the common pigeon has here been used because, as a matter of routine, in our collection of these birds we keep accurate records of the date of laying the first egg in life.

METHODS OF PREPARATION AND ANALYSIS

The samples of egg-albumen for analysis were taken from eggs immediately after laying—in one-half hour to two hours after laying. This is important since we have found (No. 2 of these studies) that the egg-yolk absorbs water from the albumen, in addition to the well known continuous loss of water from the albumen to the air. The

perfectly fresh eggs—before any trace of air space begins to form—are weighed, steamed for eight minutes, and the shell carefully removed without disturbing the shell membrane of the egg. This shell membrane forms a part of the albumen sample. The hardened yolk is then removed and weighed at once. The weight of the yolk, plus that of the shell is subtracted from the original weight of the entire egg, and this gives the true weight of the albumen sample.[1] The albumen is preserved at once in absolute alcohol.

The methods of analysis are those described by Koch[2]—a four-hour alcohol extraction, followed by a one-hour ether extraction (after which the material is very finely pulverized), and this by a twelve-hour alcohol extraction. The ash was separately determined for the alcohol-ether-soluble, and alcohol-ether-insoluble fractions.

EXAMINATION OF THE DATA

Thirty samples of egg-albumen were analyzed. These include samples obtained from the first eggs in life, obtained both in summer and winter;[3] the immediately next few eggs from these same birds were analyzed, and after an interim still other eggs were taken from the same birds. For further comparison, samples were taken from older birds—birds that had previously laid large numbers of eggs. These samples were likewise taken in both winter and summer.

The results are fully given in table 1. It is clear from an examination of these data that the albumen from the first and immediately succeeding eggs of the pigeon contains measurably less water, and more alcohol-ether-insoluble substance, than does the albumen secreted later by these same birds; and that these earliest secretions bear a similar relation to the later secretions of other (older) birds as well.

The first five eggs in life of ♀545 show an average water content of the albumen of 89.08 per cent. The average of four of the later eggs of this same bird (her 23d to 26th) was 89.61 per cent. The averages of eggs laid by other birds after numerous previous eggs are: (♀A421) 4 = 89.47 per cent, 2 = 89.93 per cent; (♀A410) 4 = 89.47, 2 = 89.25 per cent.

[1] The removal of the shell is a rather tedious process and permits an evaporation of water which prevents the direct weighings of the albumen.

[2] Koch: The Jour. of the Amer. Chem. Soc., xxxi, no. 12, 1329.

[3] Certain differences which one of us (R.) have found to obtain in the size of yolks from summer and winter eggs (or rather spring and autumn) led us to carry the observations over the two seasons.

The albumen of the first five eggs of ♀ 511 averages 89.12 per cent water. Two later eggs (her 23d and 24th) average 89.33 per cent water, More significant than the comparison of the averages of the figures obtained from these groups of earliest eggs with groups laid later is the comparison of the values obtained for the individual eggs of the two groups of earliest eggs. There are two such groups (♀'s 545, 511) of five eggs each. In both groups the first pair in life are smaller than the succeeding pair—also the smallest of the entire series of analyses—and in both groups, too, the fifth egg in life has a higher moisture value than either of the four previous eggs. Moreover, the four eggs of the initial pairs of the two groups, all show a lower moisture value than does any egg laid by these birds in their second period, and also lower than any egg laid by birds which had previously laid numerous eggs.

A pair of eggs—first in life—from a third female (A 105) gave a moisture value for the albumen of 89.27 per cent. This is a somewhat higher value than that obtained from the other first pairs in life, but is again less than the average for any bird that had previously laid eggs.

The differences in the moisture values are indeed not large, but they are so consistent as to leave little doubt of their reality. Next in interest, perhaps, to the fact of differences in first and later secretions of the gland, is the fact of the smallness of the difference. If—in the terms of another science—a gland "learns" by doing, it "learns" but little and the initial effort of this gland is in many respects a remarkable approximation to its later and most practiced efforts.

Whether there are differences in the percentages of alcohol-ether-soluble substance, and of ash in the two fractions, is perhaps not definitely answered by the data. But no differences are consistently indicated by the figures. The increase in solids is found in the alcohol-ether-insoluble fractions.

Neither season nor the variable amounts of albumen and yolk present in the various eggs seems to have any consistent influence on the percentage of water in the albumen.

CONCLUSIONS

It has been possible to compare the results of the very first functionings of a gland, with later functionings of the same gland.

The albumen secreted by the pigeon's oviducal glands, in their initial functionings, is not the exact quivalent of albumen secreted

TABLE 1

The chemical composition of egg albumen (pigeon) from the first, and from later, functionings of the oviduct

Winter season

First functionings of oviduct

NO. OF SAMPLE (ALBUMEN)	NO. OF BIRD	NO. OF CLUTCH (IN LIFE)	DATE OF LAYING	WEIGHT OF YOLK	WEIGHT OF SHELL	WEIGHT OF ALBUMEN	PER CENT H_2O	PER CENT ALCOHOL, SOLUBLE	PER CENT ALCOHOL INSOLUBLE	PER CENT ALCOHOL SOLUBLE ASH	PER CENT ALCOHOL INSOLUBLE ASH
779	545	1^{1}*	December 6, 1914	2.900	1.005	12.445	88.93	0.77	10.30	0.33	0.35
780	545	1^{2}	December 8, 1914	2.910	1.080	12.360	88.88	0.80	10.32	0.34	0.32
795	545	2^{1}	December 24, 1914	3.280	1.030	13.050	88.91	0.79	10.30	0.35	0.34
804	545	3^{1}	January 4, 1915	3.165	1.020	13.165	89.06	0.81	10.13	0.34	0.38
805	545	3^{2}	January 6, 1915	3.580	1.085	12.625	89,62	0.86	9.52	0.35	0.33
Average				3.147	1.044	12.609	89.08	0.81	10.11	0.34	0.35
778	511	1^{2}	December 3, 1914	2.860	0.981	12.799	89.00	0.80	10.20	0.32	0.36
783	511	2^{1}	December 12, 1914	2.935	1.035	13.230	88.79	0.68	10.53	0.33	0.36
784	511	2^{2}	December 14, 1914	3.150	1.000	13.305	89.24	0.73	10.03	0.34	0.32
793	511	3^{1}	December 23, 1914	2.960	0.960	12.955	89.03	0.72	10.25	0.33	0.35
794	511	3^{2}	December 25, 1914	3.182	1.002	13.338	89.36	0.70	9.94	0.33	0.36
Average				3.017	0.996	13.125	89.12	0.73	10.19	0.33	0.35

Later functionings of oviduct (of other individuals)

829	A421	$+^{1}$†	February 20, 1915	3.360	1.070	12.320	89.33	0.89	9.78	0.37	0.28
830	A421	$+^{2}$	February 22, 1915	3.750	1.170	13.260	89.44	0.88	9.68	0.38	0.28
838	A421	$+^{1}$	March 2, 1915	4.020	1.130	13.805	89.48	0.87	9.65	0.38	0.28
839	A421	$+^{2}$	March 4, 1915	4.100	1.240	13.630	89.64	0.95	9.41	0.39	0.26
Average				3.808	1.153	13.254	89.47	0.90	9.63	0.38	0.28
831	A410	$+^{1}$	February 21, 1915	3.830	1.140	13.210	89.35	0.89	9.76	0.37	0.30
832	A410	$+^{2}$	February 23, 1915	3.990	1.120	13.190	89.53	0.88	9.58	0.38	0.31
836	A410	$+^{1}$	March 2, 1915	3.220	1.095	12.745	89.62	0.90	9.47	0.38	0.27
837	A410	$+^{2}$	March 4, 1915	3.620	1.090	13.435	89.38	0.87	9.75	0.37	0.29
Average				3.665	1.111	13.145	89.47	0.89	9.64	0.38	0.29

Summer season

First functionings of oviduct

938	A105	1[1]	July	15, 1915	2.990	1.000	11.220	89.22	0.77	10.02	0.30	0.57=‡
939	A105	1[2]	July	17, 1915	2.598	1.025	11.400	89.32	0.45	10.23	0.25	0.35
Average					2.794	1.013	11.310	89.27	0.61	10.13	0.28	0.35

Somewhat later functionings of oviduct (see above)

923	545	12[1]	June	25, 1915	3.240	0.975	14.595	90.61	0.62	8.78	0.34	0.27
924	545	12[2]	June	27, 1915	3.380	1.060	13.955	89.03	0.66	10.31	0.33	0.28
927	545	13[1]	July	5, 1915	3.532	1.095	14.310	89.60	0.66	9.74	0.32	0.33
928	545	13[2]	July	7, 1915	3.620	1.120	14.067	89.19	0.64	10.16	0.30	0.28
Average					3.443	1.063	14.232	89.61	0.65	9.75	0.32	0.29
940	511	12[1]	July	19, 1915	3.970	1.060	14.310	89.27	0.73	10.00	0.38	0.34
941	511	12[2]	July	21, 1915	3.857	1.060	13.401	89.38	0.73	9.89	0.35	0.31
Average					3.914	1.060	13.856	89.33	0.73	9.95	0.37	0.33

Later functionings of oviduct

925	A421	+[1]	July	1, 1915	4.370	1.190	13.650	89.85	0.70	9.45	0.35	0.32
926	A421	+[2]	July	3, 1915	4.595	1.295	13.975	90.01	0.71	9.27	0.35	0.27
Average					4.441	1.243	13.813	89.93	0.71	9.36	0.35	0.30
942	A410	+[1]	July	19, 1915	3.394	1.075	11.171	89.33	0.81	9.86	0.39	0.35
943	A410	+[2]	July	21, 1915	3.462	1.060	11.733	89.17	0.86	9.97	0.37	0.59‡
Average					3.428	1.068	11.452	89.25	0.84	9.92	0.38	0.35

* The exponents of this column indicate that particular eggs were *first* or *second* of the clutch.

† The plus (+) sign indicates that this bird had previously laid *numerous* eggs.

‡ This figure probably inaccurate.

later by these glands. The difference concerns chiefly the relative proportions of water and solids.

Albumen produced in initial efforts contains a smaller percentage of water and a higher percentage of alcohol-ether-insoluble substance.

During a few, or several, of the earlier functionings of the oviducal glands, there probably occurs a *gradual* change from the initial lower percentage of water to the later higher percentage of water.

Partial analyses of the albumen of the pigeon's egg are recorded and one source of variation in the moisture value of this albumen has been identified.

The amounts of alcohol-ether-soluble substance, and of inorganic matter probably do not vary widely nor consistently.

The amount of water in the egg albumen of the pigeons studied shows no consistent variation in respect to summer and winter.

The earliest secretion of the albumen-secreting gland of the pigeon's oviduct is, in several respects, a rather close approximation to the later products of the gland.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

V. The Effect of Alcohol on the Size of the Yolk of Pigeon's Egg

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It is known[1] that normally in pure species of doves and pigeons a difference in size usually exists between those ova (yolks) which will prospectively produce males and females; the males more often arise from the smaller, the females from the larger ova. One of us proposed to study the effects of alcohol and other anaesthetics on sex development and control, and it seemed advisable to carry on parallel studies on the effects of some of the anaesthetics on the size of the eggs. The results here recorded are those thus far obtained with alcohol.

These data show that the ova produced with a certain amount[2] of alcohol regularly present in the blood for a considerable part of the day are smaller than the ova normally produced by the same bird. This result, and indeed any result whatever would, perhaps, have been difficult to foretell. The normal growth of the yolk (or ovum) during its last five days in the bird's ovary is made at an exceedingly rapid rate—as shown in number 1 of these studies[3]—and the materials for this growth are of course derived from the blood in competition with the other tissues of the body. Whether alcohol—which certainly modifies the metabolic rate of the tissues, and is easily soluble in the egg-yolk—will in the sum of its effects in the organism facilitate or hinder the storage processes in the egg, was the problem of particular interest to us. No data seems hitherto to have been obtained bear-

[1] Riddle. Sci., N. S., xxxv, 462; Bull. Amer. Acad. of Med., xv, No. 5, 265; Amer. Nat., 1916, l, 285.

[2] Only the particular dosage used in these experiments can now be considered; it is not improbable that a dosage producing an opposite effect will later be found.

[3] Riddle: This Journal, 1916, xli, 387.

ing on the question. Niclaux[4] and Renault have shown that alcohol has a decided affinity for the reproductive glands, even of mammals, where the proportion of fatty substance is quite small as compared with the functioning ovary of the bird. And Stockard[5] has demonstrated that alcohol influences the hereditary capacities of the treated germs of mammals.

Although the "alcoholized germs" from this series were smaller than the normal—thus resembling in size the more typical male-producing eggs—they were nevertheless probably "weaker germs"[6] than the normal. Incubation tests of several of the eggs of females 624 and 631 indicate that both of these birds produced under alcohol a predominance of females. For the reasons stated in the preceding paragraph it is not permissible to say that the smaller size of eggs produced under alcohol is an evidence of their being made more like male-producers. The size attained by the ovum under alcohol is the expression of a new metabolic adjustment between ovum and competing somatic tissues under the influence of an added factor. The smaller of the "alcoholized" eggs are—as is the case among the normal ones—probably prospectively more masculine than the larger "alcoholized" eggs. But size comparisons cannot be made directly from an alcoholized series to an unalcoholized series, since the planes or levels of body metabolism under which they were produced were probably quite different.

METHODS AND DOSAGE

The alcohol was given by inhalation.[7] The birds were placed in a glass box of 6 cubic feet capacity; this box afforded slight ventilation about the edges of the sloping lid or top (forming upper part of side as well as top), and had a $\frac{5}{8}$ inch opening at either end on a level with the floor. About $2\frac{1}{2}$ square feet of alcohol-pan surface was exposed. The amount of surface available for the evaporation of alcohol (95 per cent) into the air of the tank was further much increased by the use of large amounts of cotton-batting projecting above the surface of the alcohol. In a preliminary trial the group of birds to be tested

[4] Niclaux: L'obstetrique, 1900, Tm. xv.

[5] See note 7.

[6] This fact was established by other studies on the eggs of this "alcohol series" which were incubated, instead of opened for the measurement of the yolks.

[7] This method has been described by Stockard, Arch. Int. Med., October 1912, and Arch. f. Entw'mech., 1912, xxxv, 3.

was left in the tank without alcohol for more than five hours without showing any discomfort; with alcohol in the tank locomotion became quite unsteady or impossible in two to three hours. The period of alcoholization lasted usually one, one and one-half to two hours. With the exception of March 22, March 28 to April 2, April 7 and May 17 they were dosed daily. Four of the birds were treated for four months; three for only two months.

The females of various kinds of doves and pigeons were selected for the test—two common pigeons (*C. livia dom.*) two blond Ring doves (*St. risoria*) and three hybrids. The weights of the yolks produced by these birds immediately preceding alcoholization had been learned. Eggs laid during the alcoholization were steamed (as were the earlier ones) for eight minutes to harden the yolks which were then removed and weighed. A considerable number of eggs produced during this period were tested for fertility, longevity and sex and will not be further treated here. The yolks of several eggs, laid after the period of alcoholization was over, were taken from each of the treated females and their weights ascertained.

DISCUSSION OF THE DATA

The weights were taken of 114 yolks. Of these, 31 are from the pre-alcoholization, 45 from the alcoholization, and 38 from the post-alcoholization period. The results are presented in table 1. It will be seen that six of the seven alcoholized birds show a very notable decrease in size of yolks produced under alcoholization. The seventh (no. 384) showed a very slight increase in size. This bird was one of three alcoholized for the shorter period—only two months. In six cases also the yolks of the post-alcoholization period were notably smaller than those of the pre-alcoholization period.[8] In the single exception (no. 242) the difference between the two is slight. When a further fact[9] is taken into consideration, namely that during the progress of the season, i.e., during the alcoholization and post-alcoholization periods—the yolks of untreated birds normally become *larger*, the significance of the figures given in table 1 cannot be questioned.

[8] The ova were exposed to the effect of the alcohol while they were in their period of slow growth (see no. 1 of this series of papers), but not during the final period of four to five days of rapid growth as were those of the alcoholization period.

[9] See literature cited under note 1. The weights of the treated birds did not decrease during the alcoholization period.

TABLE 1

Effect of alcohol (March 17 to July 17, 1914) on size of egg

		♀242. Hybrid*		
Average of 4 eggs, January	4 to February	18, before	alcohol period..	1.921
Average of 4 eggs, April	7 to May	8, during	alcohol period..	*1.744*
Average of 8 eggs, May	18 to December	19, after	alcohol period..	1.956
		♀290. Hybrid*		
Average of 4 eggs January	13 to February	12, before	alcohol period..	1.915
Average of 4 eggs, March	17 to May	5, during	alcohol period..	*1.776*
Average of 8 eggs, May	23 to September	15, after	alcohol period..	1.710
		♀624. Blond Ring		
Average of 5 eggs, January	12 to March	14, before	alcohol period..	2.225
Average of 4 eggs, March	31 to April	13, during	alcohol period..	*2.110*
Average of 5 eggs, July	21 to November	2, after	alcohol period..	2.072
		♀631. Blond ring		
Average of 4 eggs, January	28 to March	11, before	alcohol period..	1.813
Average of 10 eggs, March	30 to July	17, during	alcohol period..	*1.758*
Average of 11 eggs, July	23 to December	29, after	alcohol period..	1.793
		♀646. Common pigeon		
Average of 4 eggs, January	18 to March	11, before	alcohol period..	4.003
Average of 5 eggs March	20 to July	17, during	alcohol period..	*3.209*
Average of 3 eggs, September	25 to November	26, after	alcohol period..	2.603
		♀(102). Common Pigeon		
Average of 6 eggs, February	11 to March	11, before	alcohol period..	3.728
Average of 10 eggs, March	21 to July	4, during	alcohol period..	*3.356*
Average of 1 egg, August	19,	after	alcohol period..	3.155
		♀384. Hybrid†		
Average of 4 eggs, February	10 to March	5, before	alcohol period..	1.343
Average of 8 eggs, March	21 to May	18, during	alcohol period..	*1.358*
Average of 2 eggs, July	9 to July	11, after	alcohol period..	1.145

* Alcohol discontinued May 16, 1914.

† Alcohol discontinued May 17, 1914.

CONCLUSIONS

When alcohol is given by inhalation for one to two hours daily, during a period of months, to doves and pigeons the yolks produced by these birds become smaller than during the pre-alcoholization period. This decrease occurs even during the season when the yolks of untreated birds normally grow larger than in the earlier period.

Yolks produced during a few weeks or months after the alcoholization period are smaller than normal.

It cannot be inferred that the reduction in yolk size which occurs under alcoholization renders these eggs more like the smaller eggs of the untreated doves in their *developmental capacities*. Size of yolks in the two groups is not directly comparable on this basis, since the alcohol introduces an important added factor. The evidence at hand indicates that though the alcoholized yolks are reduced in size, they are developmentally more like the larger normal eggs, than they are like the smaller normal eggs.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

VI. Sexual Differences in the Fat and Phosphorus Content of the Blood of Fowls

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In earlier studies on metabolic differences between the male- and female-producing eggs (yolks) of pigeons, it is shown by one of us (1, 2, 3, and much unpublished data), that maleness and femaleness in this earliest stage of the organism differ quantitatively in their capacity to *store* material within themselves. The substances which are there elaborated and stored—to a degree unusal in most tissue cells—are chiefly fat and phosphatides. The question naturally arises as to the continuance of this original distinction between eggs of prospectively different sex-value into embryonic and adult stages. Do similar metabolic differences characterize the adults of the two sexes? Several studies have been undertaken in an effort to answer these questions. In the present paper we present the results of a first inquiry into the amount of fat and phosphorus in the blood, in relation to sex and sexual activity, in the common fowl.

At this point it may be stated that the following data demonstrate that the blood-plasma of female fowls—in both their laying and non-laying periods—contains more alcohol-ether-soluble substance, and more phosphorus, than does the plasma of the male; and that these substances are more abundant in sexually functioning females than in owls with inactive ovaries.

METHODS OF PREPARATION AND ANALYSIS

The blood was drawn from the jugular vein through a paraffined cannula into a paraffined beaker; the bird having been previously given an injection of 2 to 2.5 cc. of a 0.1 per cent hirudin solution (in NaCl +

oxalate solution). Only local anaesthesia by cocaine was used, and this was used sparingly. The collected blood was centrifuged 2300 revolutions per minute for ten minutes, in 50 cc. haematocrits; the volume of corpuscles was recorded, and the available plasma decanted into a weighing tube, and thence to a porcelain evaporating dish. The samples were then placed for 9–24 hours—according to size of sample—at 85° in a drying oven to make them brittle, and to reduce to a volume convenient for an extraction cup.[1]

The crushed sample was at once extracted for four hours with alcohol in a Koch extraction apparatus; then for one hour, with ether, after which the sample was removed, pulverized, and returned for a twelve hour extraction with alcohol. The phosphorus was determined by the Neumann-Pemberton method on both the alcohol-soluble and insoluble fractions of the plasma. The figures for phosphorus are given in terms of lecithin (phosphatides); this is done here partly because it facilitates a comparison with the phosphorus determinations on the several kinds of tissue being studied by us, in which the phosphorus is uniformly so expressed. The methods of analysis throughout are also the same as those used in the analysis of other tissues reported upon in this series of papers.

Two samples used for preliminary total phosphorus determinations, and referred to in the text only, are of unseparated plasma and corpuscles to which sodium oxalate was added in the collection of the sample.

The egg-laying history (for one or more years) of the birds used was known from their trap-nest records. The ovary of every female killed was also examined to make certain the distinction between the laying and non-laying fowls. In every case the findings in the ovary and the trap-nest records are in agreement.

PRESENTATION OF DATA

In table 1 are given the results of the analyses of the plasma of the birds of the three groups—males, non-laying females, and of females known to be producing and laying eggs. It will be seen that the amount of alcohol-soluble substance and phosphorus is lowest in the males, clearly higher in the non-laying females, and very much higher in the laying females. The relative amounts of phosphorus in the

[1] Some oxidation of unsaturated fatty acids doubtless occurred during this period of drying.

three groups is expressed by the figures, 100 : 115 : 205. The alcohol-soluble materials are similarly expressed by the figures 100 : 116 : 181. It is clear that the proportion of alcohol-soluble substance, and total phosphorus, in the plasma of these three groups, mark them off as natural groups. The numbers are not large, but the differences are pronounced and practically conclusive. Only one of the figures within any group over-laps any figure in either of the other groups. This is for the phosphorus content of the plasma of a male, and is still far below the amounts of phosphorus found in any of the actively functioning females.

Further evidence that the non-laying condition is accompanied by low fat and phosphorus content of blood, as compared with the active egg-laying condition is to be had from two sources: (a) The third analysis (April 11, table 1) in the section for laying fowls was of a sample obtained from the bird having by far the poorest egg-laying record of any of the five of the series. Her blood proved to be markedly poorer in fat and phosphorus than the others of the series, and yet clearly richer in these substances than any of the fowls which were laying no eggs whatever. (b) The results of two preliminary phosphorus determinations on two samples of blood (plasma + corpuscles) of non-laying females supply additional confirmations of this relation. The figures obtained were 6.50 per cent and 7.23 per cent phosphatids[2] (calculated as lecithin); since there are considerable amounts of both phosphorus[3] and alcohol-soluble substance in the corpuscles of birds, and since the latter make up only about one-third to one-fourth of the total volume of these blood samples it is clear that the results of these two determinations confirm those made on the plasma alone.

Sufficient additional data are included in table 1 to show that neither age (within the limits used), nor size, nor breed of bird, is responsible for either of the three observed levels at which fat and phosphorus exist in the blood-plasma. The amount of plasma used in each analysis, and the percentage of water found in each sample are also recorded.

Of considerable interest is the distribution of phosphorus between the alcohol-soluble and alcohol-insoluble fractions; and further the variation in this distribution in the three groups of birds. A complete

[2] These percentages are not strictly accurate for total blood samples since correction has not been made for the amount of increase of total solids due to the sodium oxalate and salt solution used in collecting the sample.

[3] Ackermann (Zeit. f. physiol. chem., xliii, 302) gives the amount of phosphorus in the nuclei of the corpuscles of the hen as 3.93%.

TABLE 1

Analyses of blood-plasma from males, non-laying, and laying female fowls

Males (roosters)

DATE	DESCRIPTION OF BIRD	WEIGHT	WEIGHT SAMPLE	WATER	ALCOHOL SOLUBLE	PHOSPHATIDES*	RATIO OF P IN 3 SERIES
			gram				
April 11	Wh. Wyandotte (mong.) 2 years old	2,282	111.90	95.23	15.57	5.29	100
April 21	Wh. Wyandotte (mong.) 2 years old	2,410	88.38	94.63	15.32	8.62	
November 23	Mongrel	2,480	46.10			5.39‡	

Non-laying females

December 2	Wh. Wyandotte 1½ years old	1,405	19.90	94.48	17.46	7.90	115
December 18	Houdan 1½ years old	1,375	42.95	94.13	18.21	6.62	
December 18	Wyandotte-Houdan (mong.)? 3 years?	2,015	37.80	93.34	17.93	7.75	

Females laying eggs

April 5	Game, 3–4 years old	1,750	42.00	93.47	30.52	13.83	205
April 5	Plymouth Rock, 3 years old	1,500	47.78	93.86	28.20	14.81	
April 11	Wh. Wyandotte (mong.) 1 year old†	1,404	42.51	94.38†	21.99†	9.86†	
April 11	Wh. Wyandotte (mong.) 1 year old	1,332	48.67	94.02	33.63	14.39	
April 21	Wyandotte-Houdan (mong.) 11 months old	1,380	28.69	94.17	25.07	12.84	

* Phosphorus calculated as lecithin.

† This bird laid only two eggs during the nine days preceding the taking of the sample, the poorest laying record of any female of this series.

view of this situation is to be had by reference to table 2. The alcohol-soluble fraction contains 2.5 to 4.5 times more phosphorus than does the insoluble fraction. But it will be noted that the greatest disproportion of the two is found in the males; least in the actively laying females; and that here again the non-laying females form the intermediate group. The relation within the three groups of the distribution of phosphorus in the two fractions of the analysis, is a further evi-

TABLE 2

The distribution of phosphorus in the alcohol-soluble and insoluble fractions—(analyses of table 1)*

ANALYSIS	MALES		NON-LAYING FEMALES			LAYING FEMALES				
	April 11	April 21	December 2	December 18	December 18	April 5	April 5	April 11	April 11	April 21
Alcohol soluble....	18.87	21.84	20.80	16.71	17.61	26.10	25.92	21.38	21.80	20.00
Alcohol insoluble..	2.78	6.23	5.17	4.36	5.61	8.44	10.45	6.61	10.63	10.44
Ratio insoluble to soluble.....	1 : 4.5		1 : 3.6			1 : 2.5				

* Calculated as lecithin.

dence that the three groups of birds, are natural groups in reference to the fat and phosphorus content of the blood-plasma.

GENERAL CONSIDERATIONS

Apparently there are but few published observations bearing on the relative amounts of fat and phosphorus in the blood of males and females; we know of none which deal solely with the blood-plasma of a vertebrate. A still smaller number of published data deals with the changes in the quantity of these substances in the blood or plasma of females in relation to periods of ovarian rest and activity.[4]

Gorup-Benanez (4) makes the meager but interesting tabular statement that the blood of males contain *less* fat, that of females *more*.[5] Miescher (5) noted that at the time of ripening of eggs in the ovaries of the salmon the blood-serum contained "erhebliche Mengen" of lecithin and fatty acids. Heim (6) showed that in a number of species of Decapod Crustacea the blood of the females acquires a brilliant yellow lipochrome (alcohol-soluble) pigment at the time of the ripening of the ovaries.

The work of Geoffrey Smith upon the blood of the crab (Carcinus maenas) is, however, of particular interest in connection with the

[4] The changes in quantity of lipoids of the blood in disease, and the normal and experimentally induced fluctuations in the fat content of the blood, do not really require consideration in connection with the current topic. Some of these will, however, be considered in other of these studies.

[5] Since this paper was written a paper by Goettler and Baker (Journ. Biol. Chem., xxv, June, 1916) bearing on this subject has been published. The fat and cholesterol of male and female human (normal) blood was determined, be-

present study. Smith made adequate quantitative determinations of the total ether-soluble contents of the blood of females which were maturing their eggs. This amount was found to be much greater than that similarly obtained from the blood of the males approaching moult; and also very much greater than the colorless combined bloods of males and females. Unfortunately, perhaps, for our purpose, the blood of males and females were not compared at the period of their lowest fat content.

Smith's work has additional merit because of its elucidation of the relation of the blood changes to other carefully studied somatic changes which occur under the so-called "parasitic castration" of these forms by *Sacculina*—a condition in which the males tend to take on female secondary sexual characters, young females tend to hasten the assumption of such adult female characteristics, and the blood of both sexes becomes much richer in fat and lipochrome pigment. Additional interest pertains to these studies because, on the basis of the results, Smith has formulated a theory of sex to fit all the facts as observed in Carcinus and related crabs.

It will thus be seen that Smith has clearly recognized and studied a connection between certain aspects of sex and fat-content of the blood. Though we cannot here adequately consider the theory advanced by Smith it should be noted, that he has not assumed or sought

sides 26 other substances. The authors do not call attention to the sex differences of the fat estimations, but if we summarize and arrange their data according to sex it will be found to be as follows.

SEX	FAT			CHOLESTEROL			FAT + CHOLESTEROL
	Max.	Min.	Av.	Max.	Min.	Av.	
Man	128.0	66.4	102.1	58	17	42	(18) 141.4
Woman	320.0	145.0	199.6	44	19	29	(5) 226.0

The figures indicate milligrams per 100 cc. of blood. In addition to the above a *diabetic* male gave a fat figure of 158.8. The increase in cholesterol does not seem to keep pace with the increase in fat. It is clear that the figures of Goettler and Baker indicate a higher fat content of the blood of the human female. In connection with the division of females made in the present paper it is interesting to note that the two females whose ages were 30 and 35 years give an average of 253.5 for fat in blood; while three women of 48 to 59 years show an average of only 163.7. The *functional condition* of the *ovary* would seem to be associated with the highest fat values in the blood, whether that ovary utilizes much fat (fowl) or little (woman).

for *initial* differences in fat metabolism, but holds that the parasitic Sacculina—by depriving the blood of the male host of circulating fat as does the ovary of the female—stimulates a continual production of yolk (= "sexual formative substance") which thus accumulates in the blood, and whose presence in the blood causes the parasitized (castrated) male to assume the characters of the female. Some features of Smith's theory would seem to possess obvious advantages over some current conceptions. It remains to point out, however, that the results obtained from the study of the crab's blood— and as we believe on the general topic of 'parasitic castration' in crabs—are in entire agreement with the theory of sex which has been stated by Riddle (1, 2, 3) and that, in so far as the blood analyses contrast the blood of males and females, the results are similar to those described in this paper. No phosphorus values were obtained from the crab's blood; and the blood of the normal "inactive" male and female was not compared.

When one begins from the point of vantage stated at the outset of this paper—the demonstated initial difference in storage metabolism (particularly of fat and lecithin) of male- and female-producing ova of the pigeon—it is clear that ever new and subsidiary hypotheses are unnecessary for the general understanding of many hitherto puzzling sex phenomena. A higher fat content of the blood of woman, female crab, and hen, than of man, male crab, and cock, is, on Riddle's view, the expected result of a mere continuance or persistence of the observed relative powers of the eggs.

The eggs, which prospectively will produce organisms of the two sexes, are themselves already differentiated in respect to their fat and phosphorus metabolism[6] in precisely the same direction as are the plasmas of adult males and females. The female-producing egg is more plentifully supplied with fat and phosphorus than is the male producing egg (Riddle). The metabolic levels of the eggs accord with that abundance. The blood and plasma thus far investigated indicate that the metabolic levels of the two kinds of eggs are continued from the germ through the embryogeny into the adult.

SUMMARY

A differentiation of the fat and phosphorus metabolism of male-

[6] The proportion of *water* is also apparently higher in the plasma of males (94.93%), than in inactive females (93.98%), or actively laying females (93.98%). The eggs of different sex value are similarly differentiated.

and female-producing ova in the pigeon (Riddle) invites a comparison of the quantities of these substances present in the adults of the two sexes.

The blood-plasma of female fowls is found to be richer in alcohol-soluble substance and phosphorus than is the plasma of the male.

The blood-plasma of the sexually functioning female fowl contains more alcohol-soluble substance and more phosphorus than does the plasma of fowls with temporarily inactive ovary.

The male, the non-laying female and the actively laying female fowl are three natural groups of fowls when these are considered from the standpoint of the fat and phosphorus content of the blood-plasma. The relative distribution of phosphorus in the alcohol-soluble and alcohol-insoluble fractions of the blood-plasma is also different for these three groups of fowls.

The differences here observed and measured are quantitive not qualitative in kind.

These results on the fowl, together with the few data available on other forms, lend support to the view advanced by Riddle that the initial metabolic differences between eggs of different sex value persist in the organisms developed from the eggs of the two grades.

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